CHARLES JOSEPH CHAMBERLAIN

(WITH PORTRAIT)

The death of Professor CHAMBERLAIN on January 5, 1943, marks the passing of the last of those who, in the nineties, pioneered in establishing the Department of Botany at the University of Chicago. Dr. CHAMBERLAIN was a graduate student on the campus before any botanical courses were offered, and his insistent desire for such work was no small factor in bringing about the establishment of botanical courses and the early organization of the department. He was one of the group on hand to welcome Dr. JOHN M. COULTER in 1894, when he first made weekly visits to the campus for lectures and conferences. As Assistant and Associate, Dr. CHAMBERLAIN was in charge of the botanical laboratories from the beginning. He was the first to receive the Doctor's degree from the department (in 1897), was Instructor in 1901, Assistant Professor in 1907, Associate Professor in 1911, and Professor of Morphology and Cytology from 1915 until his retirement as Professor Emeritus in 1929. After his retirement he remained active, lecturing at various institutions and continuing his writing and his research—especially on the morphology and systematics of the cycads.

CHARLES JOSEPH CHAMBERLAIN was born near Sullivan, Ohio, on February 23, 1863. He received the usual rural school education, and attended high school in Oberlin, completing the three-year course in two years. This was followed by a year in the preparatory department and four years in Oberlin College, where he completed the classical course for the A.B. degree in 1888. He taught in the public schools for five years after graduation, including the Crookston (Minn.) high school, where he was Principal, 1889–1893.

His early attraction to botany came from interest inspired by Prof. A. A. WRIGHT of Oberlin College, a geologist who taught botany and zoölogy with equal enthusiasm. Returning to Oberlin during the summers, Dr. CHAMBERLAIN completed the requirements there in botany for the M.A. degree (1894). He was awarded the D.Sc. from the same Institution in 1923. At Oberlin he first saw the celloidin method and a sliding microtome used on animal tissues. During his first year at Chicago, zoölogical courses acquainted him with the newer techniques in animal histology and cytology. This training constituted his foundation for the adaptation of these and other methods in the study of plant tissues. His volume, Methods in Plant Histology, still the standard book of its kind, first appeared in 1901, and was enlarged and revised through five editions to 1932.

Close collaboration with Professor COULTER in teaching and research resulted in the publication of the Morphology of Seed Plants (1901), the Morphology of Angiosperms (1903), and the Morphology of Gymnosperms (1910, 1917). His books also include The Living Cycads (1919), Elements of Plant Science (1930; translated into Spanish, Buenos Aires, 1942), and Gymnosperms: Structure and Evolution (1935). His monograph on the cycads had not reached publication stage at the time of his passing, but it is so

nearly completed that it may be expected to appear soon.

His botanical researches are too numerous to mention in detail and include investigations in all major groups of plants as well as gymnosperms. The investigations of cycads at Chicago were inspired by him and the material he provided. His special interest grew out of a collaborative investigation with Dr. COULTER on the embryogeny of *Zamia*, which led to the realization of the general lack of knowledge concerning the other cycad genera, mostly tropical. While in Germany at Bonn (1901–02) for a year's research in Professor STRASBURGER'S laboratory, he learned the approximate location of *Dioon edule* in Mexico. After his return from Europe he made a collecting trip into Mexico, which resulted in the discovery of several excellent cycad localities. Four trips to that country, together with an arrangement for the periodic shipment of cones and plant material to Chicago, provided him with material of *Dioon edule*, *D. spinulosum*, and *Ceratozamia mexicana*. He visited Cuba in 1914, and again in 1922, in the study of *Microcycas*. His most extensive excursion for cycads was made in 1911–12 to New Zealand and Australia, returning via South Africa, for a study in their habitats of the remaining genera and most of the species.

These collections resulted in the assembling in the botanical greenhouses at the University of Chicago of the most extensive and complete collection of cycads ever brought together. His book, The Living Cycads, includes an account of these botanical excursions. This initial interest in cycads, and the stimulation of much research in this field, have developed the knowledge of the group, so that from the least known they are now the best known of the gymnosperms.

Professor CHAMBERLAIN was well known internationally as a foreign or corresponding member of botanical societies in England, Germany, Switzerland, India, and elsewhere. In 1923 he was Vice President and Chairman of the Botanical Section of the American Association for the Advancement of Science and was elected President of the Botanical Society of America in 1931. Among other activities, he was for many years American editor for cytology in Botanisches Zentralblatt; he was also Associate Editor of the Botanical Gazette.

As a boy, early musical talent had led his parents to expect that he might pursue music as a profession, but he contented himself with participation in orchestras, bands, and choral organizations of the college and community, a type of activity which remained an avocation throughout life. In Chicago he served for 10 years as director of music at the Woodlawn Baptist Church and was a member of the Apollo Club for 35 years. When a youth, he had learned to transcribe and arrange band music, and some of his original compositions were played by the town band. One of his musical themes was rendered on the Chapel organ at his funeral service.

During World War I, he joined in activities of the Rifle Club organized by Dr. W. J. G. LAND and soon qualified as Sharpshooter at Fort Sheridan, later as Expert Rifleman in both the Navy and unified Army-Navy courses.

Not only his family, but his wide circle of friends and colleagues, will miss him. His former students, now found in all parts of the world, gratefully remember his kindly but critical advice and counsel and will always cherish his memory as a friend. His passing is a deep personal loss to all of us.—J. T. BUCHHOLZ, *University of Illinois, Urbana, Illinois.*

VEGETATION AND SUBSTRATE OF CRANBERRY GLADES, WEST VIRGINIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 551

H. CLAYTON DARLINGTON

(WITH SEVENTEEN FIGURES)

Introduction

In several parts of West Virginia there are small isolated areas of bog or peatland ranging in elevation from 2500 to 3500 feet. The most important in size and peculiar vegetation are the Cranberry Glades in Pocahontas County. These occur in a level area of about 600 acres, at an elevation of 3375 feet, surrounded by hills ranging in elevation from 4000 to 4600 feet. Approximately 400 acres are covered with shrubs; about 80 acres with bog forest; and about 120 acres with sedges, mosses, and lichens. The latter 120 acres are popularly called "glade," four divisions of which are designated as Big Glade (approximately 60 acres), Flag Glade (28 acres), Long Glade (20 acres), and Round Glade (8 acres). Six different plant communities were recognized at the beginning of the study as worthy of investigation: bog forest, sedge-sphagnum, moss-lichen, sphagnum-cranberry-beaked rush, and two shrub communities.

The area lies in the southwestern part of Pocahontas County, about 20 miles west of Allegheny Mountain and the Virginia state line, and 9 miles west of Marlinton, the county seat of Pocahontas County (fig. 1). It is at the headwaters of Cranberry River, which flows through the bog. The area may be reached at present by turning off from Federal Route 219 at Millpoint and following the road up Stamping Creek to its head on Cranberry Mountain, the round trip totaling 12–15 miles. One then must walk 7 miles on a round trip from the top of Cranberry Mountain to the different parts of the bog.

The objects of the present investigation were: (*a*) to examine the geology of the region and the profile of the bog sediments with the hope of finding some explanation for the formation of the level bog area; (*b*) to examine the layers and the pollen of the peat to shed some light on the past history of the vegetation of the region; (*c*) to study the various ecological factors and their influence in producing the present forest, shrub, sedge, and moss communities; and (*d*) to observe successional trends and try to determine the probable future of the area. The study has extended over a period of 12 years. Previous to 1941 only two or

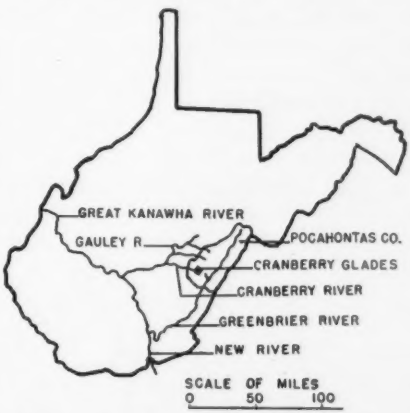


FIG. 1.—Location of Cranberry Glades, West Virginia.

three trips were made to the bog each year, but beginning with April, 1941, full time was devoted to the work until November of that year.

BROOKS (1, 2) listed some of the common plants, birds, and mammals of Cranberry Glades and estimated the approximate acreage of the different glades. He noted the importance of the area on water flow. SHELDON (11) collected and identified many species of fungi from this region. STRAUSBAUGH (12) discussed some of the bog species and also some of the rare plants of the area, such as *Cornus canadensis*.[1]

Climate

General climatic conditions in Pocahontas County can be divided into two classes, the highland climate and the low valley climate. The valleys have an elevation of 2100–2400 feet, while the highlands reach 3000–4600 feet. Winters are severe in both but are more severe in the highlands. Summers are cool, with the maximum day temperature in July rarely above 88° F. in the valleys or 80° F. in the highlands. Night temperatures may drop as low as 55° F. in Cranberry Glades in July.

While there is no complete record of climatic factors in the highlands, data have been kept for 30 years by S. L. BROWN at Marlinton (2125 feet). Temperatures throughout this paper are given in Fahrenheit and precipitation in inches. These records show a 30-year mean air temperature of 48°, with means of 67.6° and 27.7° for July and January. The maximum mean for July was 71.2° in 1898; the minimum mean, 62.3° in 1920. The highest mean for January was 34.4° in 1913; the lowest was 16° in 1918. There is no record available for the absolute maximum and minimum during this 30-year period; however, in Cranberry Glades during the summer of 1941 the maximum recorded was 82° on July 22.

The 30-year mean in precipitation is 47.28 inches. July has the highest monthly mean (5.31 inches); November the lowest (2.93 inches). The highest annual rainfall recorded was 63.62 in 1918, and the lowest was 39.54 in 1912. The rainfall is fairly evenly distributed through the months of the year, September, October, and November having the lowest monthly values and June, July, and August the highest. This is favorable to plant growth. In snowfall the 30-year mean is 31.5 inches, with 9.5 for January, 9.2 for February, 5.8 for March, 1.2 for April, a trace for May, 0.6 for October, 1.6 for November, and 6.3 for December. No snow has been reported for June, July, August, and September. The maximum annual fall was 48.5 inches for 1916; the minimum annual was 11.5 for 1913.

Mean length of the frost-free period for the 30-year period is 146 days, May 15 to October 8. The maximum was in 1919, April 19 to October 20 (183 days), while the minimum was in 1913, June 10 to September 23 (104 days). For the Cranberry Glades region it would be a close approximation to put the mean annual temperature at 42°, the mean annual rainfall at 51 inches, the mean annual snowfall at 40 inches, and the mean frost-free period at 140 days. Although not present to record the temperature, the writer has satisfactory evidence in the appearance of the plants that in 1941 there was frost on May 15 and October 2, a frost-free period of 140 days.

Physiography and geology

The area is in the Allegheny Plateau subdivision of the Appalachian Province.

[1] With the exception of lichens and mosses, the nomenclature used is that of GRAY'S New manual of botany, 7th ed.

The rocks exposed are sandstones and shales, which, with the limestone beneath, were deposited in a vast inland sea during Mississippian and Pennsylvanian time and were uplifted at the close of the Permian (table 1). The region of greatest uplift was farther east, causing all strata in this area to dip to the west.

TABLE 1

GENERAL COLUMNAR SECTION OF ROCKS EXPOSED IN CRANBERRY GLADES AREA OR LYING BENEATH IT

Era	Period	Series	Group	Thickness (feet)	Description
Quaternary	Recent			?	Unconsolidated clays and gravel
	Pleistocene			?	Unconsolidated clays and gravel
Paleozoic	Upper Carboniferous or Pennsylvanian	Pottsville	Kanawha (part)	130	Sandstones, shales, coals
			New River	500	Massive sandstones, hard dark shales, coals
	Lower Carboniferous or Mississippian	Mauch Chunk	Bluestone	75–200	Vari-colored soft shales and sandstones
			Princeton conglomerate	50– 70	Massive gray and brown sandstones with pebbles
			Hinton	500–700	White weathering sandstones, variegated shales (soft)
			Bluefield	550–800	Variegated shales, sandstones, and limestone
		Greenbrier		220–600	Limestone of various colors (gray, blue, light gray)
		Maccrady		20– 50	Red and purple shales
		Pocono		60–600	Sandstones and shales

During Cretaceous or Jura-Cretaceous time the Allegheny Plateau was eroded by water action to an almost perfect base level, the Schooley peneplain. Another uplift at the close of the Cretaceous was followed by peneplanation in the less resistant areas to the Harrisburg peneplain in Tertiary time. Black Mountain, with an elevation of 4600 feet, and other hills around the bog area are remnants of the Schooley peneplain. Still another uplift occurred at the close of the Tertiary, resulting in further dissection and erosion.

The level bog area, apparently a part of the Harrisburg peneplain, is 3 miles long and ¼–1 mile wide. It lies at an elevation of a little less than 3400 feet at the upper or eastern end and 3350 feet at the lower end (fig. 2). It is apparently level but has a drop of 45 feet in 3 miles.

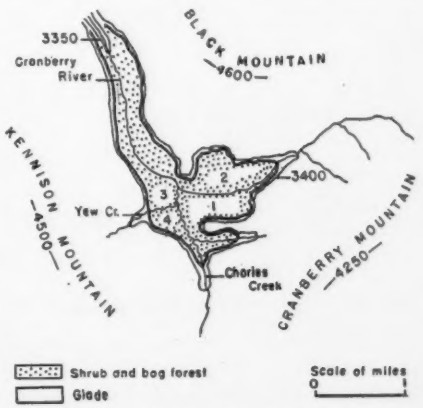


FIG. 2.—Level bog area. Dotted area shows location of shrub and bog forest of about 480 acres; white area represents open glade. Nos. 1, 2, 3, and 4 show, respectively, location of Big Glade (60 acres), Long Glade (20 acres), Flag Glade (28 acres), and Round Glade (8 acres).

Cranberry River has its source in springs on Cranberry Mountain $1\frac{1}{2}$ miles east of the bog. This stream, about 12 feet in width, flows through the bog and is joined in the lower part by Charles largest meanders (fig. 3). Feeding these streams are hundreds of cold springs on the hillsides, and especially on Cranberry Mountain, which has many more springs than Black or Kennison mountains, ow-

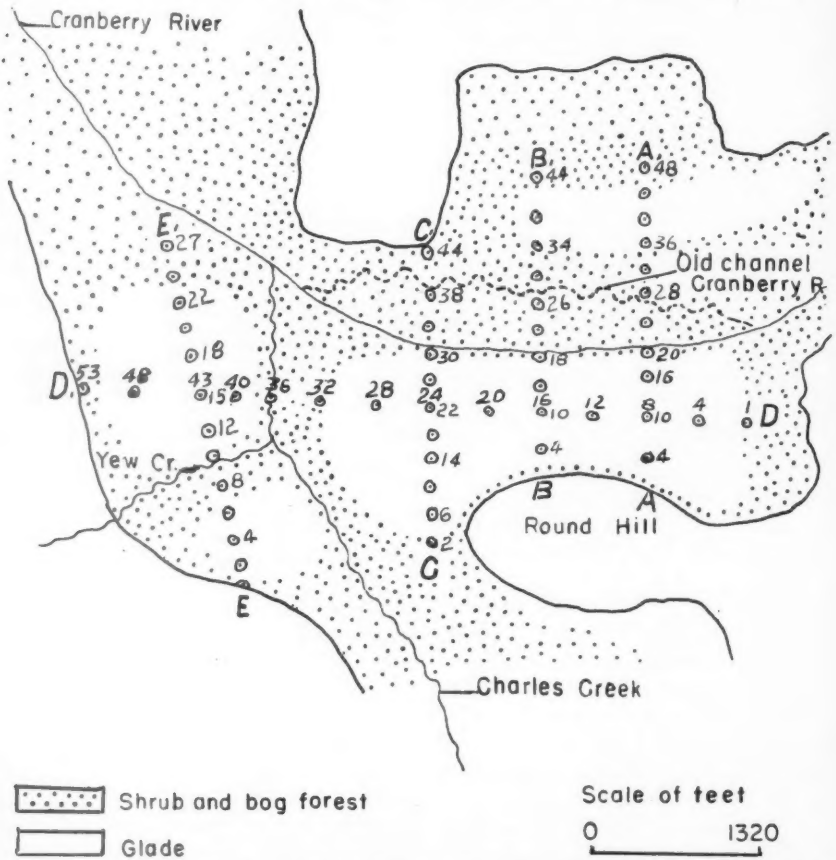


FIG. 3.—Apparently level bog area with location of transect profiles. Transect: A-A_1, with 48 borings at 50-foot intervals; B-B_1, with 44 borings at 50-foot intervals; C-C_1, with 44 borings at 50-foot intervals; D-D_1, with 53 borings at 100-foot intervals; E-E_1, with 27 borings at 100-foot intervals.

Creek, formed from small streams from both Cranberry and Kennison mountains. These streams meander extensively through the bog, with the exception of the new and almost straight course of Cranberry River along the north side of Big Glade—which has cut off some of its ing to a difference in geological formation. Cranberry Mountain is composed entirely of Mauch Chunk shales and sandstones, which in erosion produce wide flat-topped hills very receptive to rainfall and water from melting snows, thus allowing continual spring and seep-

age flow from the hillsides. Black and Kennison mountains are capped by 500 feet of New River sandstone and massive shales of the Pottsville series, which in base level, but streams 1½ miles from their spring source and 3400 feet above sea level usually cut V-shaped valleys, as Cranberry River is doing below the bogs.

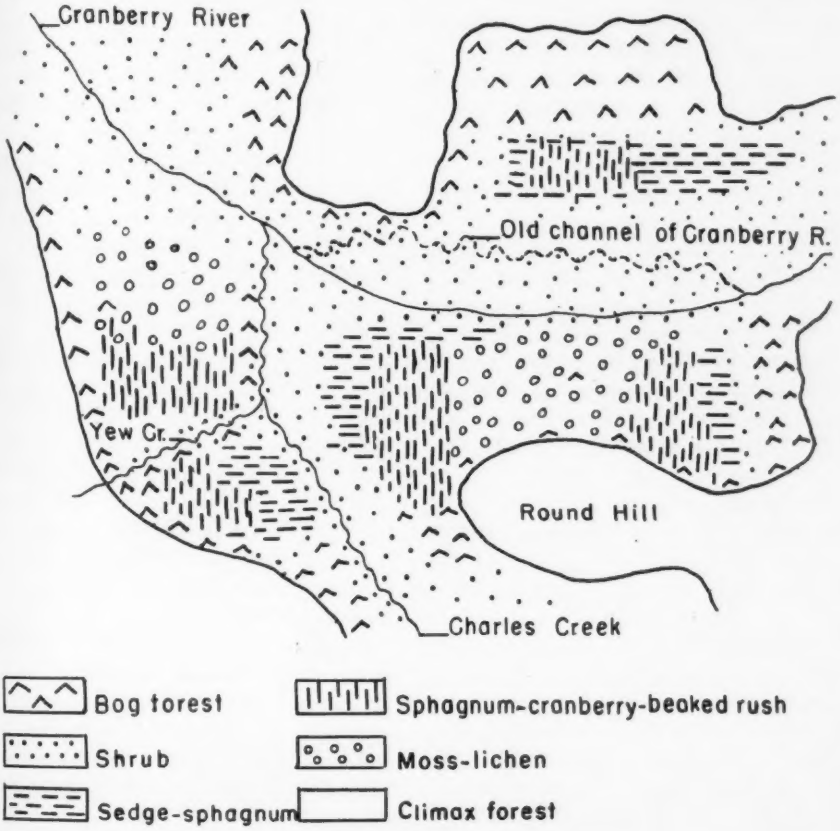


FIG. 4.—Community distribution over the area

erosion form sharp ridges not receptive to rainfall and melting snow.

ORIGIN OF LEVEL AREA

The writer early was confronted with the puzzling question of the origin of a wide valley so near the source of a stream. This is no ordinary occurrence. Mature rivers may develop wide valleys by lateral cutting when approaching So far as can be learned, no explanation of this unusual situation has been attempted other than a suggestion that a mountain lake has been filled with sediments and plant remains, thus producing the level area. Yet before a lake could have existed, the widened valley had to be formed. Two possible methods of widening were given consideration.

1. ORIGIN BY SINKING.—Solution of

limestone below shales and sandstones may cause a collapse of strata, thus forming depressions and often lakes. The fact that the bottom of the peat at the center of Big Glade (figs. 8, 9) is 8 feet lower than the bank of Charles Creek at the lower end of this glade is suggestive of a sink here. Furthermore, the Greenbrier limestone lies beneath this area at an elevation sufficiently above the valley of Hill's Creek to the south to permit underground drainage of the Cranberry Glades area to the Greenbrier River through Hill's and Locust creeks. However, the strata beneath the bog consist of at least 800 feet of shales and sandstones, which should stand up—even with solution of the limestones beneath. It is therefore improbable that there has been any collapse of strata here to form or to aid in the formation of the widened valley by erosion.

2. ORIGIN BY SURFACE EROSION.—With the uplift of the Schooley peneplain at the close of the Cretaceous, the streams began anew their work of erosion and dissection. Greenbrier River took its course southwestward with the outcrop of the less-resistant strata of the Mauch Chunk and Greenbrier series. Cranberry River and others to the north took their courses to the northwestward with the slope of the peneplain, due to warping in the uplift. The location of the head of Cranberry River is in a critical position in two respects: (*a*) it is near the beginning of the slope of the peneplain westward; (*b*) it is in a location of increased dip of strata. Measurements show that from the top of Cranberry Mountain to the center of the bog area, a distance of 3 miles, the dip is 275 feet to the mile, while for the next 5 miles downstream the dip is 125 feet to the mile. This condition caused the upper few miles of the stream to cut in mostly softer rocks, while the remainder was cutting in hard sandstones and conglomerates.

During Tertiary time Greenbrier River cut down much faster than Cranberry River because it was cutting in soft rock while the latter was cutting in very resistant sandstones and conglomerates. By the close of the Tertiary, Greenbrier River had cut down to near the bottom of the Greenbrier limestone, 2100 feet below the Schooley level, and had formed a partial peneplain (Harrisburg) in the less resistant area—as evidenced by the Hillsbora plain, Jerico Flats, Key Flats, and other points. During the same time, Cranberry River and its branch, Charles Creek, had cut down about 1200 feet. The upper parts, cutting in soft strata, reached temporary base level in the Mauch Chunk shales, the level being controlled by the hard layers downstream. Sideward cutting in soft rock then allowed the streams to widen their valley to these unusual widths. It is to be noted, however, that they were considerably longer then than now and were gradually shortened by the rapid downward cutting of Greenbrier River and the piracy of its branch, Stamping Creek, westward into the drainage basin of Cranberry River and its branch, Charles Creek, thus moving the divide farther westward. The widened valley is thus a part of the Harrisburg peneplain, the writer believes, although it is 1000 feet above the Hillsbora level.

Another uplift at the close of the Tertiary caused the Greenbrier to entrench 400 feet into the Harrisburg peneplain to the present level. No such entrenching occurred in the Cranberry Glades area, owing to the slow cutting of the river in the very resistant conglomerate below the level area. Widening probably continued, but more slowly, as the rejuvenation of the branches of Greenbrier River

led to further piracy and shortening of Cranberry River and Charles Creek.

Soils

Soil of surrounding hills

The profiles of the bog were examined carefully, and some attention also was given to the soil of the surrounding area to the top of Kennison Mountain, 1100 feet above the bog. The soils of Cranberry Mountain and the lower parts of Kennison and Black mountains are derived from mixtures of the many-colored shales and sandstones of the Mauch Chunk series with more or less humus. On Cranberry Mountain the soil is deeper and contains more humus than on Black Mountain, because of less exposure to the sun, more spring and seepage flow, and fewer fires. Kennison Mountain is intermediate in these respects. Soil tests for acidity in the surrounding area show a range from pH 4 in spruce forest on top of Kennison Mountain to pH 6.2 on Cranberry Mountain in maple-birch forest. No neutral or alkaline soil was found.

Soil of bog

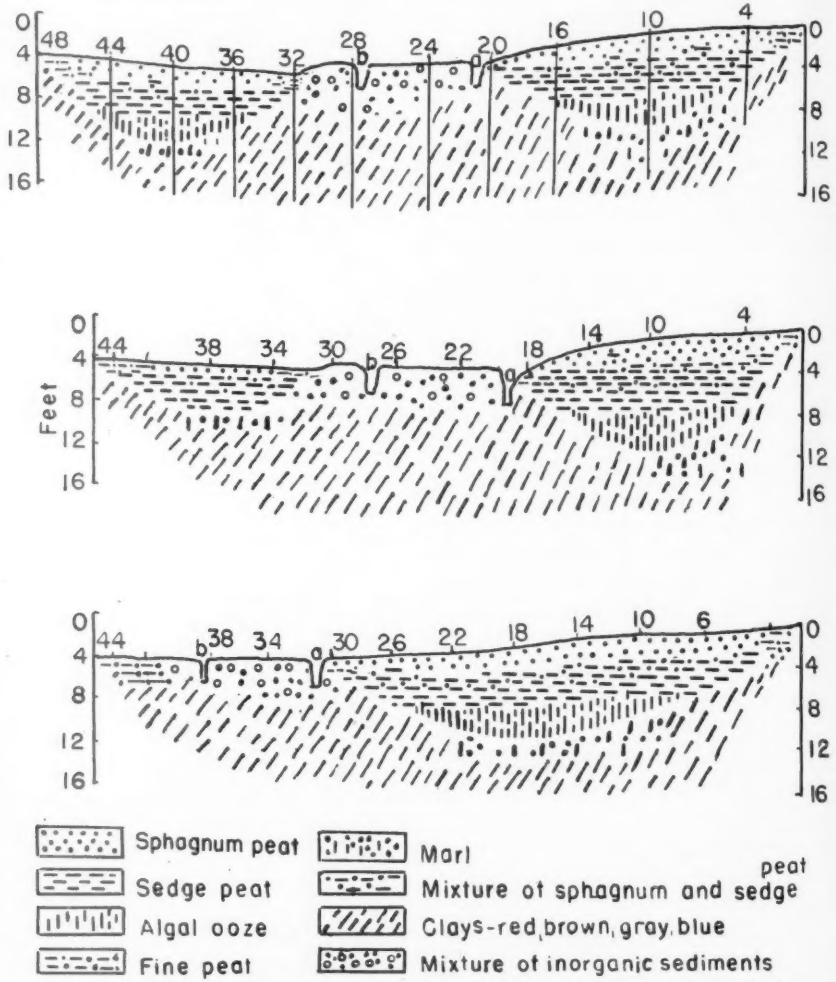
Figure 3 shows the location of many profile samples along various transects in the area. All parts of the bog have been sampled in order to obtain a clear picture of the accumulated sediments and peat. More than 150 borings were made, with a Hillier peat-borer.

Transect A.—This transect passes from Round Hill on the south directly north across the middle of the upper half of Big Glade, across a belt of alders along Cranberry River, and across Long Glade into spruce forest north of Long Glade, a distance of 2400 feet (fig. 3). Borings were made at intervals of 50 feet to varying depths. Generally samples were taken as deeply as the borer could be pushed into the very stiff compact clay. Fifteen feet was the maximum depth from which it could be recovered by two men.

The profile revealed by this transect is shown in figure 5. Stations 1–20 show the profile of Big Glade, beginning at its southern margin at Round Hill. Station 1 is 50 feet inside the margin of the bog and shows 4 feet of fine peat with many logs. Below this are 2 feet of clay and sand underlain by rock. Between this station and the base of the hill several other borings showed that the peat thinned to 1 foot at a distance of 3 feet from the base of the hill and was only 1 foot above rocky material. Station 2, 100 feet from the base of the hill, shows 5 feet of fine peat overlying 3 feet of a mixture of clay and organic material underlain by very compact clay. Stations 1 and 2 are within a belt of shrubs, about 125 feet wide. Station 3 is 25 feet from the border of the shrub belt, in the glade. Samples taken here show 6½ feet of peat, including 1½ feet of sphagnum peat at the top, 3 feet of sedge peat mixed with sphagnum peat, 1 foot of sedge peat, and 1 foot of algal ooze. Below the peat are 2 feet of compact sticky clay. The next eight borings show similar profiles, with a gradual thickening of the peat to 8½ feet at the midway point across Big Glade. Station 11, 15 feet in depth, shows respectively from top downward 2 feet of sphagnum peat, 2½ feet of sphagnum and sedge peat mixed, 2 feet of sedge peat, 2 feet of algal ooze, 3 feet of marl, and 3½ feet of clay. The next nine stations show a similar profile, with gradual thinning of the peat to 2 feet at station 20 at the border of the shrub belt on Cranberry River. Underneath the peat is clay as far as samples could be taken. Station 21 is on the north side of Cranberry River,

and a boring made with a 2-inch pipe to a depth of 11 feet shows only fine sediments of clay and sand.

5 feet deep. It may overflow its banks during floods and deposit inorganic sediments. As a result, stations 21–27 show



FIGS. 5–7.—Fig. 5 (above), transect profile A. Fig. 6 (center), transect profile B. Fig. 7 (below), transect profile C. Horizontally, 1 inch equals 530 feet; vertically, 1 inch equals 16 feet. Vertical exaggeration is approximately 33. *a*, present location of Cranberry River; *b*, old channel.

From Big Glade, transect A crosses Cranberry River and a belt of shrubs, mostly alders, 660 feet wide between Big Glade and Long Glade. Cranberry River is about 12 feet wide here and in flood is

a profile of fine sediments, mixed with organic materials in some places. They were very difficult to bore. Stations 29–34 show peat 2–5½ feet in thickness, with clay beneath, as in stations 1 and 2.

Stations 35–44 are in Long Glade, with a profile similar to those in Big Glade, although the peat is only 7 feet thick at the deepest part. Stations 45–48 extend through a belt of alders on the north side of Long Glade and into spruce forest. Here the peat is 4 feet thick at station 44 and thins to 1 foot at station 48. Underneath the peat are 3–4 feet of red clay from the red Mauch Chunk shales on the hills to the north.

TRANSECT B.—This transect, beginning at Round Hill on the south side of Big Glade (figs. 3, 6), crosses an alder belt 120 feet in width, passes through the center of Big Glade, and then crosses a shrub belt to slightly north of the western end of Long Glade. The total length is 2200 feet. Stations 1–3 are similar to 1–3 in transect A. Stations 4–18 are similar to 4–20 in transect A, except that the peat is 2 feet thicker on the average and the thickness of the algal ooze is greater. Stations 10 and 11, near the center of Big Glade, show the deepest peat in any part of the entire area, 11 feet in thickness. Clay and sand extend below the peat 5 feet or more. At station 10 samples were taken for pollen analysis (figs. 3, 6, 13). Temperature readings (fig. 12) were taken here also. The different layers represented are: 2 feet of sphagnum peat, 3 feet of sedge and sphagnum peat mixed, 2 feet of sedge peat, 4 feet of algal ooze, 3 feet of marl, and 1 foot or more of clay.

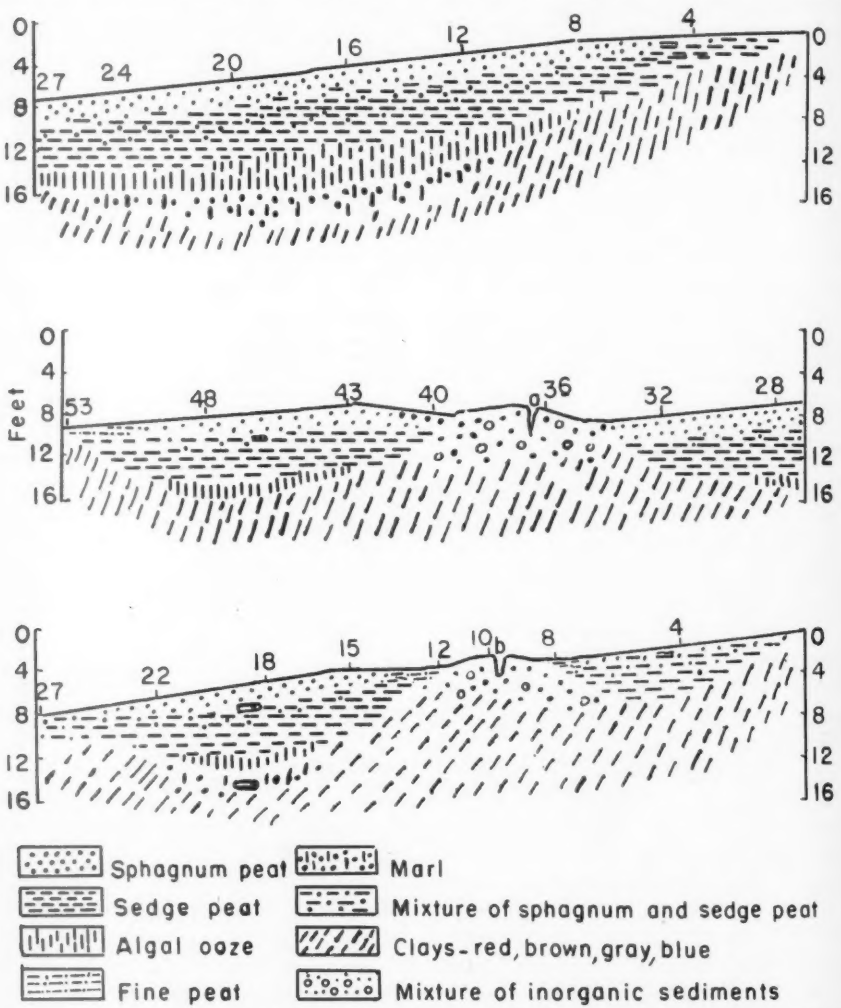
This area, showing the greatest depth of sediments, appears to be a sink in the general surface underlying the peat and supports the theory of formation of the level area by solution of limestone beneath shales and sandstones. Since it is unlikely that there has been any sinking, however, the depression will be explained by another process in a later section.

TRANSECT C.—This cuts across Big Glade in its lower part (figs. 3, 7). There are forty-four stations 50 feet apart, a total length of 2150 feet. It extends from the alder belt on the south side of Big Glade to the foot of Black Mountain. The section across Big Glade is similar to the corresponding section of transect A, with the exception of a 6-inch greater average thickness of peat. Stations 31–38 extend across the floodplain between Cranberry River and its old channel and reveal a profile of inorganic sediments similar to stations 21–28 of transect A. This area is practically level. Stations 39–44, which are north of the old channel, exhibit 1–1½ feet of fine peat accumulation. Station 30 of this transect, as well as 18 and 20 of transects B and A, respectively, shows that Cranberry River is now eroding into the peat of Big Glade.

TRANSECT D.—This is the longest transect, extending from the upper end of Big Glade in almost an east-west direction the full length of Big Glade, the belt of alders on Charles Creek, the width of Flag Glade, and the belt of alders and spruce west of Flag Glade to the foot of Kennison Mountain, a total length of 5200 feet (figs. 3, 8, 9). There are fifty-three stations at 100-foot intervals. It crosses transects A, B, and C at their deepest parts, and its profile is similar to theirs, with a gradual thinning of the peat toward either end of Big Glade but with almost a uniform thickness from transects A to C. The profile of the shrub belt on Charles Creek is similar to that of the other alder zones. In Flag Glade the peat is thinner than in Big Glade, not more than 8 feet thick at any point. The top surface of this profile shows a drop in elevation of 8 feet in the length of Big Glade, about equal to the drop in Cranberry River, which parallels it. There is a rise in elevation of 1 foot from the lower end of Big Glade to the bank of

Charles Creek. The lowest depth of peat in station 20 of this transect is 8 feet lower than the banks of Charles Creek at the lower or west end of Big Glade and 4 feet lower than the south bank of Cranberry River on the north side of Big Glade.

TRANSECT E.—This transect (figs. 3, 10) extends from the south side of Round Glade in a slightly northwest direction across Round Glade, the shrub belt on Yew Creek, Flag Glade, and into the alder zone north of Flag Glade. It includes twenty-seven stations at 100-foot inter-



FIGS. 8–10.—Fig. 8 (above) and fig. 9 (center), transect profile D. Fig. 10 (below), transect profile E. Horizontally, 1 inch equals 584 feet; vertically, 1 inch equals 16 feet. Vertical exaggeration is approximately 36.5. *a*, location of Charles Creek; *b*, of Yew Creek.

vals, totaling 2600 feet. Stations 2–7 show the profile of Round Glade, with the thinnest accumulation of peat of any of the glades, not exceeding 4½ feet. The peat is mostly sedge peat. Near the banks of Yew Creek the profile is similar to those cutting through the other streams, chiefly of inorganic sediments. The profile in Flag Glade is similar to that of Big Glade, but the peat does not exceed 8 feet at any point. The surface of the peat in Flag Glade shows a drop of 5 feet in elevation from the upper end at station 11 near Yew Creek to station 27 at the lower end.

WATER TABLE.—At the intersection of transects B and D in the center of Big Glade (fig. 3), a pit 5 feet deep was dug in 1934. The water level was read on a stake with an inch scale, driven into the pit, from April 12 to November 11, 1941 (fig. 11). On April 12 the water stood 1 inch below the peat surface, the highest recording at this station. On October 25 it was 12 inches below the surface, the lowest level recorded. This pit (designated as *A*) was in a *Polytrichum-Cladonia* community. A similar pit (*B*) was dug in Long Glade at station 38, transect A, in a *Sphagnum-Vaccinium-Rynchospora* community, and readings were made during the same period. Here the water level was 2 inches above the peat surface on April 12, and 7½ inches below on October 25. The water table was 2–5 inches higher continually than in pit *A*. Observations were made on the water table in the shrub belts along Cranberry River and Charles Creek. Pit *C* was located at station 31 of transect A in an *Alnus-Viburnum* community, and the shifting level was very similar to that of pit *B*. The lowest water table is between Cranberry River and its old channel on transect A, with a fluctuation of from 8 to 30 inches below the surface.

During 1935, 1937, and 1938, observations were made on the water table at irregular intervals. It is generally lowest in September and October.

SOIL TEMPERATURE.—At station 10, transect B, temperatures of the bog were taken from April 12 to November 11: at the surface of the peat beneath the moss, at the 6-inch depth, at the 1-foot depth, and at intervals of 1 foot down to a depth of 10 feet (1 foot above the bottom of the peat). A soil thermometer was used which was fastened to a pole for the lower levels. It was pushed into the peat to the required depth and left for 12–15 minutes, then pulled up quickly and the temperature recorded. Readings were taken between 2:00 P.M. and 4:30 P.M.

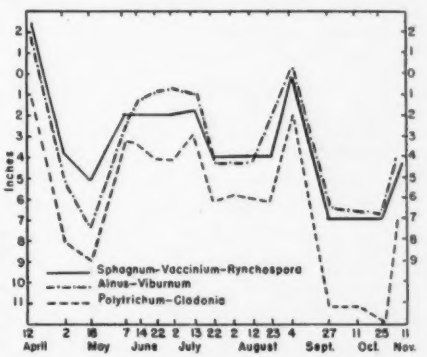


FIG. 11.—Fluctuations of water table in three communities; zero mark represents surface of peat underneath growing moss.

The surface temperature varied from 32° in April to 68° in August, while in the lowest level it rose gradually from 46° in April to 49½° in October (fig. 12). The low levels are little affected by the increase of heat on the surface through the summer. They reach their maximum temperature more than a month after the surface has reached its maximum and has begun to decline rapidly. Early in October these lower layers are still gaining in

heat and do not begin to show a net loss until the middle of October. Values for any time. The 5-, 6-, and 7-foot levels were 3°–6° colder in winter and 2°–5° warmer in summer than the 10-foot level.

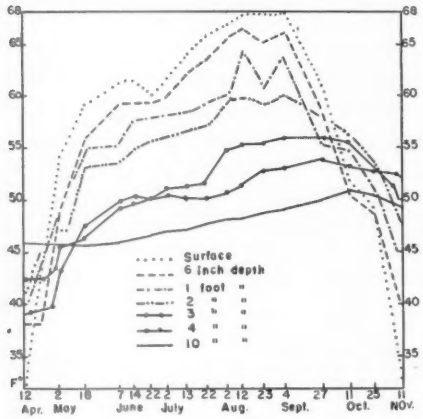


FIG. 12.—Temperature of bog profile at station 10 of transect B.

The temperature of the bog soil is no doubt influenced by the water from hundreds of cold springs which flows into it, especially from Cranberry Mountain (fig. 2). The temperature of the spring water changes little during the 12 months (44° to 54°). This water tends to maintain uniform temperature in the bog throughout the year, especially in the lower levels.

Temperatures of the profile were checked in other communities. Only the surface temperatures were found to vary from those of the above profile. The surface of a profile with a higher water table will show 1°–2° lower temperature than one with a lower table after both are exposed to the sun for several hours.

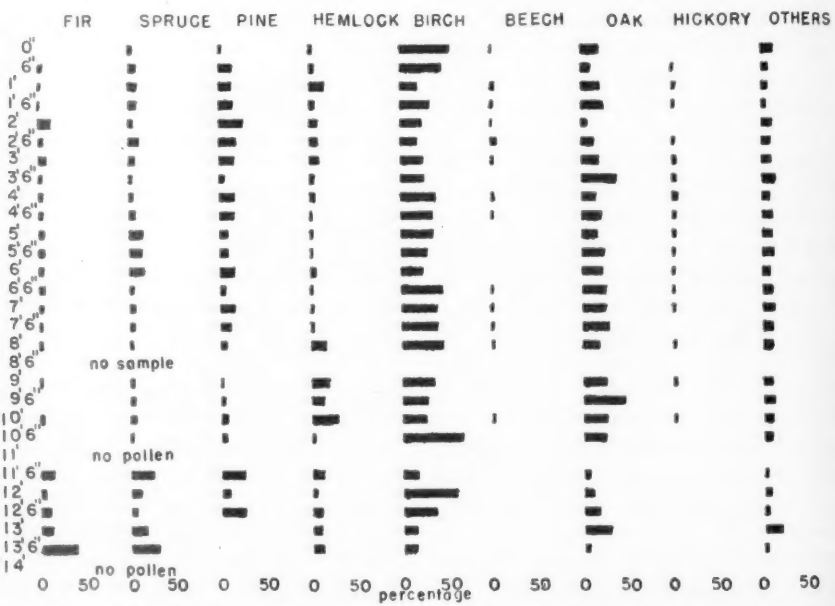


FIG. 13.—Pollen diagram at station 10, transect B. (Pollen analysis of samples by PAUL B. SEARS)

the 5–9 foot levels are omitted in figure 12. The 8- and 9-foot levels did not vary from the 10-foot level more than 1° at

SOIL PH.—At station 10, transect B, the pH profile was also determined colorimetrically to a depth of 10 feet. The values, beginning at the surface, are: surface and 6-inch levels, pH 3.8; 1- and 2-feet, pH 4; 3-, 4-, and 5-feet, pH 4.4; 6-, 7-, and 8-feet, pH 4.6; 9- and 10-feet, pH 4.8. The peat, although slightly less acid at the lower levels, is still very acid at 10 feet. KURZ (5) found in Illinois bogs a decrease in acidity with increase in depth. This high acidity in the lower levels may be caused, as KURZ suggested, by sphagnum remains in the peat or by the downward movements of water. The pH of the surface layers was also determined in the various plant communities, and the results are given in the community descriptions.

POLLEN PROFILE.—At station 10, transect B (which is also station 16, transect D, at the center of Big Glade), samples of the sediments were taken at 6-inch intervals from the surface to the 14-foot level. The pollen diagram (fig. 13) shows the results. The 13½-foot level shows a spruce-fir dominance. From that level to the surface the spectra are similar to the surface or present level, with the exception of the absence of fir at the surface level. The 12-foot level in marl and the 8-foot in algal ooze show little difference from the present level, indicating that all the sediments from the 13-foot level to the surface were deposited while the surrounding vegetation was similar to the present.

Vegetation

REGIONAL CLIMAX

The variation in altitude (3400–4600 feet) allows the persistence of three climaxes: northeastern coniferous or boreal forest, transition forest, and eastern deciduous forest. On Round Hill at 3550 feet a quadrat study shows the following, based on stem count: 70 per cent *Acer saccharum*, 4 per cent *Fagus grandifolia*, 10 per cent *Betula lutea*, 6 per cent *Prunus serotina*, 5 per cent *Tilia americana*, and 5 per cent *Prunus pennsylvanica*.

At 4100 feet on Cranberry Mountain the climax is a transition forest of yellow birch, sugar maple, beech, spruce (*Picea rubra*), and hemlock (*Tsuga canadensis*), with *Ostrya virginiana* as a smaller tree and *Corylus rostrata* as the most common shrub. A quadrat study shows 30 per cent *Betula lutea*, 33 per cent *Acer saccharum*, 20 per cent *Picea rubra*, 6 per cent *Fagus grandifolia*, 5 per cent *Tsuga canadensis*, and 6 per cent *Ostrya virginiana*.

Above 4200 feet the maple loses its dominance to spruce. On Kennison Mountain at 4500 feet a quadrat study shows 65 per cent *Picea rubra* and 30 per cent *Betula lutea*. *Abies fraseri* has not been found in this area with the spruce and birch, although in other parts of the state it does occur with these two species (3). *Taxus canadensis* is often found in this area as a low shrub. The range of mountains, including Black and Kennison, is known as "Yew Mountains" because of its abundance. The elevations where spruce dominates are somewhat lower than the 5000-foot lower limit in Tennessee and North Carolina, 400–500 miles farther south. Soil factors as well as climatic ones may have some influence on the zonal limits. *Populus tremuloides* and *Prunus pennsylvanica* have been observed as chiefly occupying a burned-over area.

VEGETATION OF BOG

The entire level area of 600 acres popularly called Cranberry Glades may properly be classified as bog. Peat covers most of the area, varying in thickness from a few inches to 11 feet. The entire

area is acid, ranging pH 3.8–6.2. The soil of all communities where sphagnum or other moss grows is near pH 4 and about pH 6.2 where no moss grows. All the area except a part of the shrub communities is partially covered with sphagnum, and there are a number of genera of the heath family, such as *Andromeda*, *Menziesia*, *Vaccinium*, and *Gaultheria*. The water table is within an inch of the surface most of the year and sinks to 12 inches below only during dry periods. This water in the partially decayed vegetable matter gives a quaking effect to the area wherever the peat is 5 feet or more in thickness. Carnivorous plants are represented by *Utricularia cornuta* and *Drosera rotundifolia*.

ORIGIN OF BOG CONDITIONS.—RIGG (7) mentioned two ways that bogs may develop: (*a*) in lakes and (*b*) in swamps. The popular belief is that this area was once a large mountain lake. Suggestions of dam formation by beavers or by landslide have been offered as possible causes of the lake. As the work progressed, this theory appeared inadequate for explanation of the existing conditions. During the last year of study, after checking the levels of the area, the lake theory of origin was abandoned as improbable if not impossible for three reasons: (1) The elevation of the lower end of the bog area is 45 feet lower than the upper end, at both the surface and the bottom of the peat, and the width of the area at the lower end is 400–500 feet. A dam 45 feet in height and 500 feet in length would have been required to flood the entire area, the construction of which would have been beyond the power of beavers or such landslides as could have occurred here. (2) If the whole area had been flooded, the deepest peat and certainly the deepest algal ooze and sedimentary peat should be found toward the lower end of the area; this is not the case, for the deepest peat and algal ooze is toward the upper end (figs. 6, 8). (3) If the origin had been in a lake, the peat deposits should cover the whole area, even under the streams. The profiles show chiefly inorganic sediments under all the streams. The peat accumulations thin out toward the streams and do not extend under them.

Another theory, which will better explain the existing conditions, attributes the damming to stream action. The maps and profiles (figs. 2, 3, 5–10) show that the main peat accumulations are located in the angles of the junctions of the streams. This led the writer to the theory that the conditions giving rise to peat accumulation owed their origin to the streams. Apparently the whole area, described previously as a part of the Harrisburg peneplain at the close of Tertiary time, was 10–15 feet lower than at present. It had been widened and base-leveled by the then longer streams while the river farther down was cutting in much harder rocks. The slope of the area may at that time have been less than the 45 feet in 3 miles shown at present. Following this period there came a change from degrading to aggrading action, and inorganic sediments were deposited as levees along the stream courses, thus damming areas away from the streams in which water could accumulate. Some explanation must be found for the change from degrading to aggrading action. This could have happened possibly during the later part of the glacial period, when increased precipitation, decreased evaporation, and lesser vegetation cover on the slopes caused the swollen streams to be overloaded with sediments, which were deposited over the level area. The coarser sediments would be deposited nearer the stream courses as levees, and the finer

clays would settle out later in the ponded water in the depressions away from the streams. The marl and algal ooze may have been deposited during this period while the climate was too cool for many aquatic seed plants. During the post-glacial period the levee building continued, but more slowly because of less precipitation, more evaporation, better vegegation cover, and a shortening of the streams through further piracy by Stamping Creek. The coming of a warmer climate speeded up plant growth in the ponded water, filling it with peat and initiating the sedge swamp.

Figure 3 and the transect profile A (fig. 5) show that the old channel of Cranberry River was building a much wider levee on its left bank. This was due to a bend in the stream to the right, so that most of the overflow was on the left. This bend also was one probable cause of the change of course later to the south side of the levee and along the margin of the peat accumulation in Big Glade, explaining the wide extent of inorganic sediments now on the right bank of Cranberry River and its erosion into peat on its left bank, both of which had puzzled the writer for several years.

Levee building, which thus probably occurred through part of the glacial and post-glacial periods, has now probably ceased in most parts of the bog. Cranberry River has recently cut through the very resistant Princeton conglomerate and is now cutting in the soft Hinton strata for several miles below the glades. Since the stream there has a drop of almost 100 feet to the mile, it should cut down rapidly, thus rejuvenating the streams of the glades. There is evidence that this is occurring in the recent shifting of Cranberry River from its old meandering channel to a nearly straight course along the side of Big Glade (fig. 3).

This theory explains all the conditions existing at present, including the sink in Big Glade referred to earlier. The bottom of peat here is 8 feet lower than the bank of Charles Creek. This could have been a former stream bed, 3–4 feet lower than the surrounding area, permitting a deeper ponding of water. The profiles show that the present surface of peat in some of the glades is higher than the levees which assisted in its origin. This is the condition of the "raised" bog (7). Once peat accumulation is started, it may form its own dam owing to slow movement of water through it and can develop on a slope, especially in cool climates (4). Parts of Flag Glade on transect D show this condition, as do also parts of Big Glade. The levees produced the undrained condition which was necessary to start bog development.

SHRUB COMMUNITIES.—These communities cover approximately 400 acres and extend as a wide belt along the streams and around the margin of the glades (figs. 4, 14). On the basis of soil, moisture, and floristic composition two units may be recognized, designated the *Alnus-Sambucus* and *Alnus-Viburnum* communities.

The *Alnus-Sambucus* community occurs on the levees or floodplains near the streams, where the water table varies from 1 to 30 inches below the surface. The soil is alluvium with some humus. The soil reaction is pH 6–6.2. The chief shrubs are the alder and elder, although some others are found. A quadrat study shows 70 per cent *Alnus incana*, 20 per cent *Sambucus canadensis*, 2 per cent *Ilex monticola*, 2 per cent *Viburnum dentatum*, 3 per cent *Taxus canadensis*, and 3 per cent *Crataegus* sp. In some parts of the community scattered trees occur, including *Fraxinus nigra*, *Prunus sero-*

tina, *P. pennsylvanica*, *Betula lutea*, *Tsuga canadensis*, and *Picea rubra*.

Many herbaceous plants occupy the ground stratum of this community, their societies giving seasonal aspects to it. *tus*, *A. prenanthoides*, *Trautvetteria carolinensis*, *Aconitum uncinatum*, and *Chelone glabra*. As a part of the aspection should be included woolly aphids on *Alnus incana*, forming great white masses

FIGS. 14–17.—Fig. 14, *Sphagnum-Vaccinium-Rynchospora* community with sedge, shrub, bog forest, and climax forest of Kennison Mountain in distance. Fig. 15, *Polytrichum-Cladonia* community in Big Glade with patch of *Andromeda glaucophylla* and shrub belt along Charles Creek. Round Hill is on left and Kennison Mountain on right. Fig. 16, shrub and bog forest invading *Polytrichum-Cladonia* community in Flag Glade. Shrub is chiefly *Viburnum cassinoides*, which aids in hummock formation. Kennison Mountain at back. Fig. 17, young *Picea rubra* in *Polytrichum-Cladonia* community. This spruce, 5 feet in height, had horizontal roots 9 feet long between moss mat and peat surface.

The vernal societies include *Claytonia caroliniana*, *Viola cucullata*, and *Ranunculus septentrionalis*. The estival societies include *Phlox maculata*, *Thalictrum* sp., *Veratrum viride*, *Ribes prostratum*, *Senecio aureus*, and *Carex scoparia*. The autumnal aspect is given by *Aster panicula-*

2 feet in length on the branches and visible at half a mile. These occur through June, July, and August.

The *Alnus-Viburnum* community is found around the margins of all the glades. The elevation is about 12–18 inches lower than the *Alnus-Sambucus*

community, and the water table varies from 2 inches above the surface to 7 inches below. Surface soil reaction is pH 4.4. *Sphagnum palustre* grows over much of it, and there is an accumulation of peat or organic material to a depth of from a few inches to 5 feet. The total area is greater than that of the *Alnus-Sambucus* community. A quadrat study shows 40 per cent *Alnus incana*, 25 per cent *Viburnum cassinoides*, 7 per cent *Ilex verticillata*, 8 per cent *Amelanchier oligocarpa*, 8 per cent *Pyrus melanocarpa*, 4 per cent *Pyrus americana*, 5 per cent *Menziesia pilosa*, and 3 per cent *Hypericum densiflorum*. Seasonal aspects also occur under the shrubs in the vernal societies of *Caltha palustris*, *Symplocarpus foetidus*, *Ranunculus septentrionalis*, and in the estival societies of *Polemonium van bruntiae*, *Arisaema triphyllum*, *Thalictrum* sp., *Oxalis acetosella*, *Osmunda cinnamomea*, *O. regalis*, *Rosa carolina*, and *Sium cicutaefolium*. The main factor differentiating these two communities is the difference in the depth of water table and possibly the amount of organic material of the soil. The difference in acidity is due chiefly to sphagnum, which is absent from one community.

CAREX-SPHAGNUM COMMUNITY.—This community extends around the eastern and western margin of Big Glade and the eastern margin of Flag Glade. It also occupies most of Round Glade, the upper half of Long Glade, and the margins of the remainder of Long Glade (figs. 4, 14). The main species of sedge is *Carex rostrata*, which extends throughout the community and makes up at least 90 per cent of the total sedge population. In some parts of the community rather extensive stands of *Carex trisperma*, *C. scirpoides*, and *C. lurida* occur. The sphagnum here is mostly *S. capillaceum*, with some *S. palustre*. This community includes also more or less of *Rubus hispidus*, *Vaccinium macrocarpon*, and *V. oxycoccus*. Little aspection is obvious after growth of sedge begins in the spring.

From 1 to 6 feet of peat underlie this community. The water-table level and pH value, 4.4, are close to those of the *Alnus-Viburnum* community, which borders it and is invading it, and to those of a *Sphagnum-Vaccinium-Rynchospora* community, which also borders it. In some places *Carex rostrata* has encroached upon the latter community as much as 15 feet in 8 years through growth of its underground rootstocks. In other places the ecotone of the two communities has not changed in 8 years; in others sphagnum has spread into pure sedge areas. Since 1–2 feet of sphagnum peat overlies 4–5 feet of sedge peat in much of the glades, sphagnum must have grown upon and killed out the sedges which first formed the peat. It is difficult to understand how sphagnum could kill out *Carex rostrata*, since the latter can invade sphagnum areas and by means of its rootstocks adjust itself to any building up of peat by sphagnum. The sedge or sedges which first formed the peat may have been acid-intolerant, existing for a long period in the absence of sphagnum and acid. With the invasion of sphagnum and the induced change in environment, they succumbed.

SPHAGNUM-VACCINIUM-RYNCHOSPORA ASSOCIATION.—This community includes a total of 50 acres in the upper and lower ends of Big Glade, the lower half of Long Glade, the upper one-third of Flag Glade, and a part of central and western Round Glade (figs. 4, 16). The percentages of each of the components of this association would be difficult to determine. *Sphagnum capillaceum*, *S. palustre*, *Vaccinium oxycoccus* (small cranberry), and *Rynchospora alba* (beaked rush) are present throughout the area. *Rubus hispidus* and *Vaccinium ma-*

crocarpon may or may not be present and in varying abundance. Through the summer the beaked rush gives the meadow aspect, which is mo ified in some parts by an abundance of two species of orchids (*Pogonia ophioglossoides* and *Calopogon pulchellus*); the autumnal aspect is given by *Eriophorum virginicum* (cotton grass), with large cotton-like masses of fruit. Cotton grass extends more or less through all the communities, giving the autumnal aspect to the whole area except the shrub and bog forest communities. *Aster umbellatus*, *Dulichium arundinaceum*, and *Epilobium densum* also add to the autumnal aspect of this association in Long Glade and in some other parts.

The reaction of the soil is pH 4. The water table varies from 2 inches above the soil surface to 7 inches below during droughts. Much of the time the water table is at the surface of the partially decayed sphagnum material, permitting the live sphagnum to be filled with water continually. The water-table level is one of the main factors determining this community, which is always located in the poorly drained areas. The upper end of Big Glade is nearly level and receives water from seepage, springs, and small streams from Cranberry Mountain; the lower end is nearly level and has its water table raised somewhat by overflow from Charles Creek, which is more than 1 foot higher. The upper end of Flag Glade is almost level and receives water from overflow and seepage from Yew Creek and springs from the hills. The lower half of Long Glade is lower than the levee or floodplain of the old channel of Cranberry River, which extends close to the protruding hill on the west and cuts off drainage by its levee at that point (fig. 3). Soil temperature is possibly another factor in determining this community. Temperature at the surface is always 1°–3° lower than in the *Cladonia-Polytrichum* community. This difference in temperature is partly due to the higher water table and partly to the influence of cold springs. This association is being encroached upon by *Carex rostrata* in some places and by *Polytrichum strictum*, *P. ohioense*, and *P. commune* in others—where the water table is being lowered.

POLYTRICHUM-CLADONIA ASSOCIATION.—This community is limited chiefly to Big Glade and Flag Glade and occupies about two-thirds of each. It occurs in the part of Big Glade between the *Sphagnum-Vaccinium-Rynchospora* association on the east and west sides of this glade and also in the northwestern part of Flag Glade (figs. 4, 15). The total area is 50–60 acres. This association includes *Polytrichum strictum*, *P. ohioense*, *P. commune*, *Cladonia rangiferina*, and *C. tenuis*. There may be more or less of *Rubus hispidus* and *Eriophorum virginicum*, the latter giving the autumnal aspect to the association. The aspect changes with the development of lichens. During periods of rainy weather and high water table, *Cladonia* grows very rapidly, spreading over whole clumps of *Polytrichum*, so that much light gray color is mixed with the green. During long periods of drought the lichen is deprived of water and dries so that it can be crumbled to powder, while the moss with long stems extending to the moist peat or organic soil then will grow around and over it.

The reaction of the soil surface in this community is pH 3.8, increasing somewhat at lower levels. The water table is 3–6 inches lower than in the communities surrounding it. The lower water table and the lower pH have the same cause. The association in Big Glade is higher than Cranberry River on its north, and it lies along the north side of Round Hill. This deprives it of any water from seep-

age, springs, or streams, leaving rainfall its only source—which is void of mineral bases capable of neutralizing the acidity. The *Sphagnum-Vaccinium-Rynchospora* association at the upper end of this glade receives the same amount of rainfall and in addition some water from the hills, thus producing a higher soil reaction and a higher water table. Similar conditions exist in the *Polytrichum-Cladonia* association in Flag Glade, also occurring on "raised" peat. It is higher than Charles Creek on its east and slopes toward the hill on the west and so is deprived of telluric water and bases, resulting in a low pH and a low water table. Wind sweep may also have some effect in determining this community. The central part of Big Glade and lower part of Flag Glade are most exposed to the winds moving through the valley. This would tend to lower the water table and increase transpiration, favoring *Polytrichum* rather than sphagnum.

The encroachment of sphagnum and *Polytrichum* upon each other is determined by the height of water table, by the amount and frequency of rainfall, and by the rate of evaporation. During periods of much rainfall and high relative humidity, sphagnum will grow over *Polytrichum*, cutting off its light and killing it. In many parts of the association this competition may be observed through the season. Where the water table is being lowered, as along Cranberry River on the north side of Big Glade, *Polytrichum ohioense* and *P. commune* are encroaching upon the *Sphagnum-Vaccinium-Rynchospora* association.

Three colonies of *Andromeda glaucophylla* have become established in the *Polytrichum-Cladonia* association in Big Glade. One large and two small colonies cover nearly an acre (fig. 15). It has greatly increased its area in 10 years, suggesting that it was introduced by bird dissemination of seeds from some other bog. A similar case is found in the *Sphagnum-Vaccinium-Rynchospora* association, where two colonies of *Menyanthes trifoliata* have established themselves, one in Long Glade and one in Big Glade, and have spread greatly in 10 years. If they have been here for a long period, they should be more widely distributed over the area.

The *Polytrichum-Cladonia* community is being encroached on all sides by shrubs from the *Alnus-Viburnum* community. *Hypericum densiflorum* has driven a wedge into the association from Cranberry River on the north side of Big Glade. *Pyrus melanocarpa*, *Ilex verticillata*, and *Viburnum cassinoides* are scattered through much of the association.

Hummocks are found in many parts of this association, usually with the shrubs (fig. 16). As mentioned before, wind movement is strong, increasing evaporation, so that at times in the summer the water table descends to 12 inches below the surface of the soil. Growth of *Polytrichum* is thus slowed down many hours and days during the summer. Those moss plants growing under a small shrub with its protective canopy of leaves, such as *Viburnum cassinoides*, will remain more moist, with resultant longer growing periods, so that they elevate themselves above the surrounding moss. *V. cassinoides* can adjust itself to the upward growth of the moss by developing adventitious roots. One plant was observed with such roots 18 inches above the original ones. Other shrubs than *V. cassinoides* also assist in hummock formation. After a hummock is once started, the shrub may die and the hummock continue to grow, mostly on its leeward side.

A study was made of the roots of many of the bog plants. Most of them were in

the *Polytrichum-Cladonia* association. None of the shrubs scattered through this association are able to penetrate the acid peat but extend their roots horizontally on the surface or above it in the living moss mat. A specimen of *V. cassinoides* 3 feet in height had four horizonal roots 8–9 feet in length. *Hypericum densiflorum* 12 inches high had one horizontal root 6 feet long and two shorter ones. *Andromeda glaucophylla*, *Pyrus melanocarpa*, and *Gaultheria procumbens* of similar heights had roots of corresponding lengths. A red spruce 5 feet in height had twelve horizontal roots 6–9 feet in length (fig. 17). None of these roots had penetrated the peat vertically more than 1 inch. The plants are held up by the moss and could not exist without it. Only four of the species were able to penetrate the peat more than 1 inch. These were *Eriophorum virginicum*, *Rynchospora alba*, *Menyanthes trifoliata*, and *Carax rostrata*. One plant of *E. virginicum* 2 feet high had roots extending vertically almost 2 feet. As previously mentioned, it is found over the whole area except in the shrub and bog forest communities and gives the autumnal aspection to the glades. Vertical roots of *C. rostrata* were found up to 18 inches long. *R. alba* penetrates the peat 6 inches with several vertical roots. *M. trifoliata* in the *Sphagnum-Vaccinium-Rynchospora* association had roots 18 inches deep in the sphagnum material. It probably will extend its area in this association.

BOG FOREST ASSOCIATION.—This community, covering about 80 acres, is on the periphery of the entire bog area (figs. 14–16). Red spruce and hemlock are the most common species. A quadrat study shows 75 per cent *Picea rubra*, 12 per cent *Tsuga canadensis*, 8 per cent *Betula lutea*, 5 per cent *Fraxinus nigra*. The shrubs include *Ilex verticillata*, *Viburnum cassinoides*, *Rhododendron maximum*, and *Taxus canadensis*. Sphagnum grows over much of the forest floor. The soil reaction is pH 4.5. The water table is high, only slightly lower than in the *Alnus-Viburnum* community, which forms an irregular ecotone with the bog forest and is being encroached upon by it. The soil is organic and varies in depth from 1 to 5 feet of peat.

In addition to invasion of the *Alnus-Viburnum* community, the bog forest is also invading the moss-lichen and sphagnum-cranberry-beaked rush communities, mostly in Flag Glade and Round Glade. Spruce is the only tree species invading the moss communities, except one specimen of *Pinus strobus* and three or four of *P. rigida* which have attained a diameter of 6–8 inches in Big Glade. It is very difficult for these trees to invade the moss communities, since their roots cannot penetrate the peaty soil and must run horizontally under the moss, so that they are soon casualties to the strong wind-sweep in the glades. If they can develop in a group of four to eight, they support one another. Many such groups occur in Big Glade and in Flag Glade, but show a slow rate of growth.

A study was made of the rate of growth of young spruce on Kennison Mountain, in the bog forest, and in the moss-lichen community of the open glade. Trees 2.5 inches in diameter were cut 1 foot from the soil, and annual rings were counted. The specimen from Kennison Mountain showed an age of 19 years; the one from bog forest, 49 years; and the one from the moss-lichen community, 31 years. The bark was equal in thickness on the mountain and bog forest specimens but was more than twice as thick on the glade specimen. The tree in the bog forest had two important limiting factors—difficulty of absorption and lack of sunlight. The one in the open glade was limited in absorption of water

and nutrients and was exposed to strong winds. With equivalent rate of growth, some of the spruces in the clumps in Big Glade 8 and 10 inches in diameter would be 125 years or more in age.

Only a few spruce seedlings are encountered in the open glade, showing that establishment there is very difficult (fig. 17). The migration is chiefly from the west, in the direction of the prevailing winds, which carry the seeds. The recently uprooted spruces in the glades, the trunks now being covered with mosses and lichens, and the countless trunks encountered at all depths to 13 feet in the borings, all attest the attempts at invasion of this area for hundreds of years, perhaps thousands, and the magnitude of the casualty list.

Discussion

The present study has revealed much about the origin of the level area and the origin of the bog conditions, yet there are many questions unanswerable relative to past history and age. It is very difficult to estimate the length of time represented by the 15 feet of sediments which have been studied. They are certainly of Quaternary origin. They probably represent the later part of the glacial and post-glacial periods.

The pollen diagram shows that spruce and fir were dominant regionally at the time of the accumulation of the 13½-foot level of marl (fig. 13). This might seem at first to make the bog comparable in age with the Ohio and Indiana bogs, which are estimated at 30,000 or more years (6), and showing the same forest sequences mentioned by SEARS (9). The 13½-foot level would thus represent the cool-moist or spruce-fir period; the 11–12½-foot levels, the warmer and drier or pine period, etc. The altitude here is 2800–4000 feet higher than at the Indiana and Ohio bogs, however, and the spruce-fir dominance undoubtedly persisted longer. The time since spruce-fir dominance in those areas has been estimated at about 10,000 years (8). The rate of peat formation has been the basis for this estimation (10). In the area of this study the rate of peat formation has probably been slower than in the other bogs mentioned because of lower temperature and differences in origin and continuation of bog development.

The clays and marl probably were deposited in the later glacial period, and perhaps a part of the algal ooze was formed then—or in the early post-glacial period while it was yet too cool for many aquatic seed plants. Most of the peat probably was formed in the post-glacial period.

When the depressions were first formed by the levee building of the streams, water accumulated, especially in the Big Glade area. For a long period it was too cold for much plant life, so that clays and marls and later algal ooze accumulated; then, as the climate became warmer, increased plant life filled the water and initiated the sedge swamp, which formed the 5 feet of sedge peat now overlying the 4 feet of algal ooze and sedimentary peat. Some shrubs were scattered through the sedge (*Hypericum densiflorum*, for example), remains of which are now found in the sedge peat. The sedge community later retrograded to sphagnum over most of the glade area. Sphagnum has recently been giving way to *Polytrichum* in the more raised and better drained parts.

Trees have grown in the bog area since the sedge swamp was initiated and probably for some time before. Logs of spruce and hemlock are encountered to the bottom of the peat. Those in sedimentary peat or algal ooze could have been washed in by stream overflow. No logs have been encountered below the 13-

foot level. Shrubs have probably been growing along the streams since the time of deposit of the 13-foot level of sediments.

The future of the area with its successions and time required is as difficult to surmise as is its past. Succession is taking place, as shown by observations over the last 10 years. Shrubs are encroaching upon the moss-lichen, the sphagnum-cranberry-beaked rush, and the sedge-sphagnum communities. Trees are encroaching upon the shrub and other communities. Where shrubs or trees persist, their leaves and needles accumulate to raise the level higher above the water table and the acid substratum. At present, where a spruce or a clump of spruces have established themselves, many shrubs—such as *Menziesia pilosa*, *Viburnum cassinoides*, *Pyrus melanocarpa*, and *Gaultheria procumbens*—are thriving and spreading out from this area into the moss-lichen community.

In 1934 stakes were set up at a number of places on the border of different zones where encroachment seemed probable. During the succeeding 8 years *Gaultheria procumbens* has spread 10 feet in all directions from a clump of spruces into the *Polytrichum-Cladonia* association, or $1\frac{1}{2}$ feet per year. *Pyrus melanocarpa* advanced 25 feet, or 3 feet per year, into the same association. *Hypericum densiflorum* advanced 17 feet from the shrub belt on Cranberry River at transect B into the moss-lichen community. An old settler, who had often visited the area years ago but not in the last 20 years, stated that 30 years ago there were no shrubs in the Big Glade but only moss-lichen "tundra" with cotton grass and beaked rush. He was much surprised in 1941 to see the encroachment by shrubs from all sides. *Pyrus melanocarpa* is making more headway than any of the other shrubs. It has an underground rootstock which grows rapidly under or through the moss layer and sends up new shoots. Yet its roots cannot penetrate the peat and it depends upon the moss for anchorage. It will not be able to survive without the moss until the soil has been built up by the decay of leaves and other plant parts, or better drainage is otherwise provided.

At another staked area, *Carex rostrata* advanced upon a sphagnum-cranberry-beaked rush community about 15 feet during the 8 years. The sedge has underground rootstocks which provide a good mechanism of encroachment similar to that of *Pyrus melanocarpa* and *Gaultheria procumbens*. Sphagnum spread several feet into a pure *Carex rostrata* community and an *Alnus-Viburnum* community. It can spread rapidly owing to its ability to absorb water and minerals through any part and to propagate vegetatively from many of its parts.

The bog forest is encroaching on every side of the area, but mostly on the west, the direction of the prevailing winds. Dispersal of conifer seeds should be greater from the west, and the young trees are better protected from excessive evaporation and uprooting.

It is only a question of time until the shrubs will cover the whole 600 acres, gradually to be replaced by bog forest. Once it is established, it should remain as an edaphic climax, until the streams are entrenched so that the peat can be eroded and provide a drained soil for the regional climax forest. The rate of downward cutting of the streams is greatly increasing, owing to the fact that Cranberry River, which has been cutting in the hard Princeton conglomerate below the bogs, is now cutting in the soft Hinton strata. The new course of the river along the north side of Big Glade is evidence that entrenchment and peat erosion are now occurring.

Summary

1. The Cranberry Glades area in West Virginia comprises about 600 acres of apparently level land covered with bog forest, shrub, and areas of sedges, mosses, and lichens, commonly called "glade." Most of the area is underlain by peat or organic material from a few inches to 11 feet in thickness.

2. The level area, upon which 15 feet of inorganic material and peat have accumulated, represents a part of the Harrisburg peneplain temporarily base-leveled in the soft shales and sandstones of dipping strata.

3. Bog conditions were initiated by the deposition of levees along the banks of streams, causing undrained conditions in the areas away from the streams. This change from degrading to aggrading action probably occurred during glacial time, when the streams, swollen by increased precipitation and decreased evaporation and overloaded with sediments from slopes unprotected by sufficient vegetation cover, deposited their sediments over the area. In post-glacial time the streams with reduced volume of water slowly built their levees higher during floods, causing further damming and further peat accumulation.

4. Six plant communities are differentiated: sedge-sphagnum, sphagnum-cranberry-beaked rush, moss-lichen, bog forest, and two shrub communities.

5. The structure and pollen of the transect profiles of the sediments and the existing communities point to succession from shallow water, with its accompanying communities, to a sedge swamp, to a retrogression to sphagnum, to moss-lichen in some parts, to shrub, to bog forest.

6. Succession will continue until bog forest has occupied the whole area, to be followed by regional climax forest only when sufficient stream erosion has removed the peat accumulation and provided sufficient drainage.

The writer greatly appreciates the helpful direction and encouragement given by Dr. CHARLES E. OLMSTED, University of Chicago, under whose supervision the work was done. Sincere thanks are also expressed to Dr. P. D. STRAUSBAUGH who suggested the problem for study; to Dr. G. D. FULLER who offered many suggestions and help on the problem; to Dr. DAVID KIRBY who accompanied the writer into the bogs several times and gave assistance in the study; to Dr. P. B. SEARS who made the pollen analysis; to Dr. E. L. CORE who identified several sedges; and to Dr. L. E. ANDERSON who identified some of the mosses and lichens.

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SURVEY OF ANATOMY, ERGASTIC SUBSTANCES, AND NUCLEAR SIZE IN ECHINOCYSTIS MACROCARPA AND CUCURBITA PEPO

FLORA M. SCOTT

(WITH TWENTY-THREE FIGURES)

Introduction

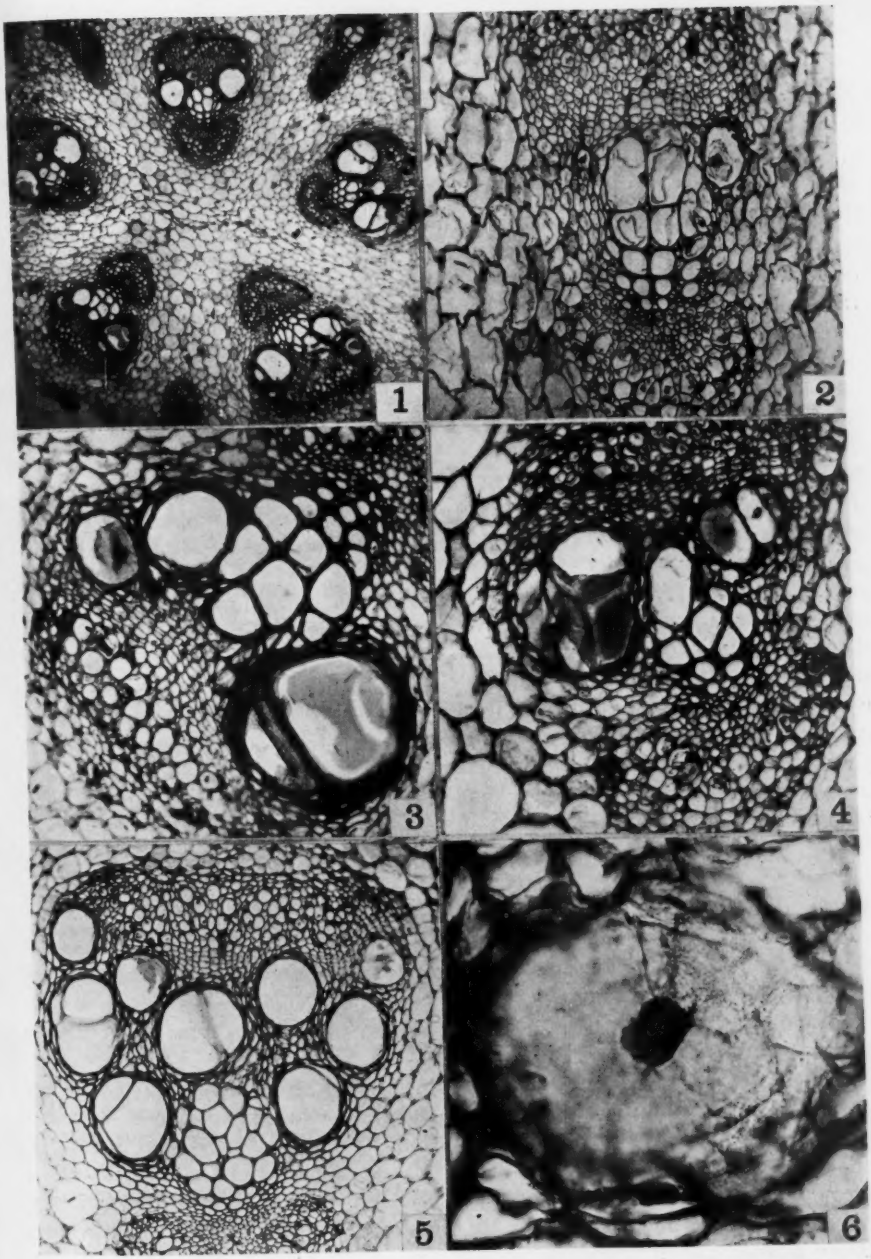
In the course of anatomical and cytological studies of *Ricinus communis* (**8**, **9**), the lack of detailed knowledge of nuclear size in differentiating xylem vessels was apparent. In contrast to nuclei measuring 59,550 cu. μ in *Ricinus*, nuclei measuring 330,023 and 163,643 cu. μ, respectively, are present in the developing vessel segments of *Echinocystis* and *Cucurbita*. Nuclear size in other tissues is surveyed for the sake of comparison, and, since apparently no report has appeared on the subject, the anatomy of *E. macrocarpa* is outlined.

Material and methods

Echinocystis macrocarpa and *Cucurbita pepo*, of the family Cucurbitaceae, are vigorous scrambling or trailing vines (**7**). *Echinocystis*, a perennial, common in the chaparral of southern California, has an immense, completely buried storage tuber. In the first two seasons' growth this "root" is about the size and shape of a small carrot. When fully grown it is rough and gnarled on the surface and may measure 20–40 cm. in diameter, 50–90 cm. in length, and may weigh 40–60 lb. In early spring, one to eight vigorous shoots appear above ground, grow rapidly to a length of 60–90 cm., then branch profusely. As the lateral branches develop, secondary thickening in the main axis is most active. During this stage, which is of relatively short duration, differentiating vessels are present in every internode of the main axis. By the time of flowering the majority of the vessels are mature and without nuclei. Full-grown shoots may measure 250 cm. or more. Lateral branches continue to grow throughout the early summer, but since they are of smaller diameter, they are much less favorable for nuclear study.

Sections of fresh material of both *Echinocystis* and *Cucurbita pepo* (variety Kentucky) were cut from the younger and older tissues of shoot, root, and developing fruit and mounted directly in mineral oil or in water containing traces of vital stains such as neutral red or thionin. Nuclei were measured immediately. No trace of shrinkage is indicated in oil mounts for several hours, but in water, surface wrinkling of the nuclear membrane may occur within a few minutes or after a longer period. Permanent slides, necessary for tissue detail, were prepared by fixation in Navashin's solution followed by Delafield's haematoxylin or safranin and fast green stain. Feulgen's reagent was also used to intensify the chromatin network.

The percentage water by volume in the giant nuclei is estimated by observing volume contraction during and after irrigation of the section with absolute alcohol (**9**). Centrifugation of tissues, followed by immediate killing and fixing, results in a shrinkage even more pronounced. Accurate measurement in this case is impossible, however, because of the piling up of plastids and cytoplasm around the shrunken nucleus.



FIGS. 1–6.—Transections: Fig. 1, young stem; two or more large secondary vessels differentiated in inner bundles. Fig. 2, young bundle; left and right of primary spiral vessels (still unlignified) lie two secondary vessels, one small and one large, in early stage of differentiation. Figs. 3, 4, bundles; different stages of differentiation. Fig. 5, mature bundle; vessels empty, none tylotic. Fig. 6, differentiating vessel; giant nucleus already somewhat contracted.

Observations

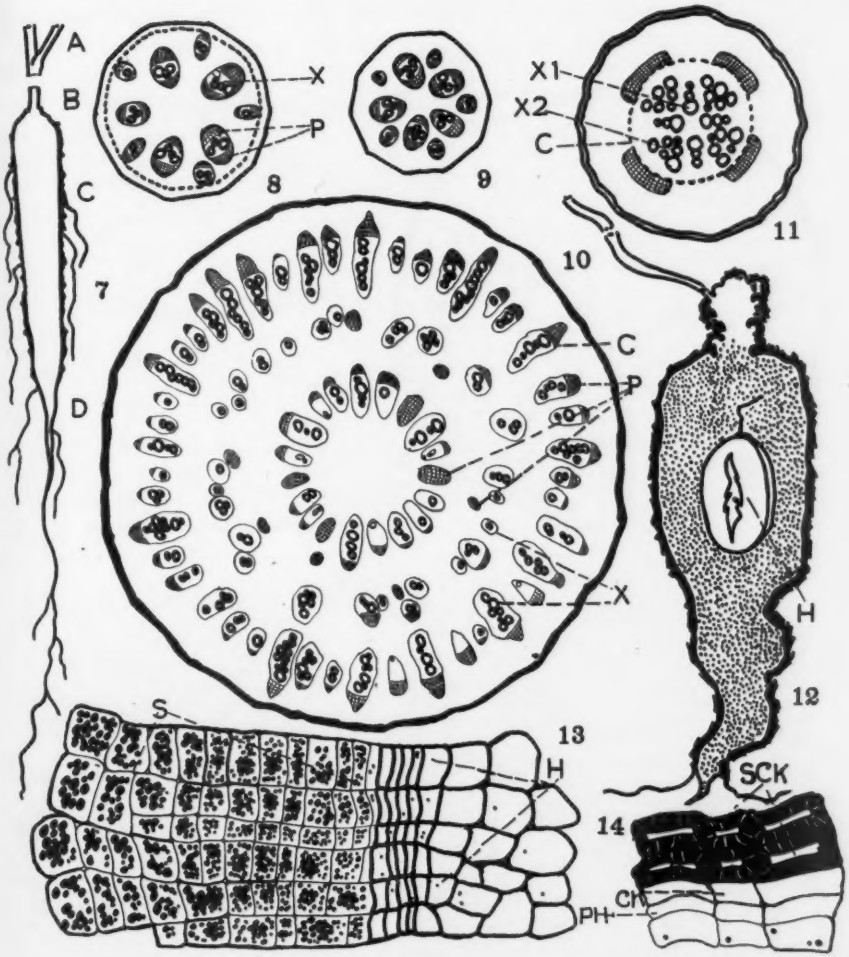
A. Echinocystis macrocarpa

Shoot.—The structure of the shoot resembles essentially that of *Cucurbita* recently summarized by Hayward (4). The entire surface, including buds, leaves, tendrils, and inflorescence primordia, is closely covered with pointed trichomes of varying length and by capitate glands. The developing axis, phyllotaxy 5/13, may be divided anatomically into four regions: (*a*) The apical meristem, surrounded by leaf and tendril primordia, is followed by twelve differentiating internodes 0.05–0.2 cm. in length. (*b*) In the zone of primary growth the internodes expand and elongate rapidly and measure approximately 0.3, 0.5, 1, 1.5, 2.5, 4, 6, 11, 18, 15, 17, 17 cm. The stem as seen in typical transection consists of epidermis, collenchyma, chlorenchyma, pericycle, and nine or ten bicollateral bundles arranged in two concentric rings in a ground tissue of ray and pith parenchyma. The primary xylem strands consist of five to seven radial rows of spiral vessels (figs. 1, 2), which are followed by scalariform and reticulate vessels. (*c*) About the level of the twenty-fifth internode the young stem reaches its maximum diameter and the lateral branches their maximum length. Secondary vessels, two or more per bundle, made up of very wide, short, pitted vessel segments now differentiate. They may appear at the same time and keep pace in expansion and in wall lignification, but more generally the development is not contemporaneous. One vessel may be completely lignified while the second is beginning to expand (fig. 3). In the twenty-seventh and proximal internodes the solitary vessels typical of the distal region may be replaced by a group of two to four differentiating vessels in the same stage of growth (fig. 4). (*d*) The mature region of the stem is usually bereft of leaves and all but the remains of tendrils; it narrows toward ground level and below. While growth of the shoot as a whole progresses, a few secondary vessels continue to differentiate (fig. 5).

Region (*c*) affords the best material for study of the giant nuclei. In any cross-section, two to eight or more differentiating vessel segments may be observed. During vessel expansion the living cytoplasm contains innumerable vacuoles (figs. 6, 20*E*). Mitochondria are numerous and chloroplasts are present, usually parietal in position. Protoplasmic streaming is frequently observed. Wall thickening may be followed from a barely visible cellulose mesh to the completely lignified, mature pitted wall, and the end walls eventually disappear.

The leaves and tendrils of *Echinocystis* are essentially similar in structure to those of *Cucurbita* (4). Since nuclear size is conservative throughout the tissues, the latter are not described in detail.

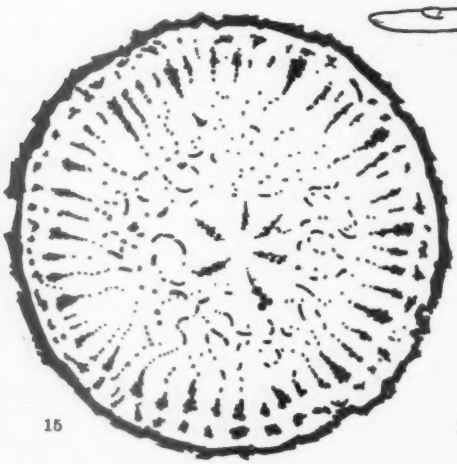
Tuber.—Owing to lack of seedling material, the early stages of tuber development were not observed. The storage cylinder of a typical plant 2–3 years old measures about 15 cm. in length and 1.5 cm. in diameter (figs. 7–11). It tapers off above and below toward shoot base and tap root. Numerous lateral roots are present throughout the tuber length (fig. 7). Since the transition to root structure occurs near the base of the storage region, the tuber presumably develops from the hypocotyl and stem base (figs. 7*D*, 11). The surface is covered with a thin layer of cork followed by phellogen and periderm. The pericyclic zone consists of fibers and parenchyma. The vascular bundles are arranged in three roughly concentric series and are surrounded by starchy parenchyma. The



FIGS. 7–14.*—Fig. 7, diagrammatic longisection of young tuber (actual diameter 1.5 cm.); length of storage region 15 cm.; length of shoot (etiolated) below ground 5 cm. General structure of shoot at ground level (*A*), at junction of shoot and tuber (*B*), of midstorage region (*C*), and of transition region (*D*) are illustrated in figs. 8–11. Fig. 12, diagrammatic longisection of mature tuber with cracked central heart devoid of starch; actual diameter and length 26 cm. and 60 cm.; actual diameter and length of heart 10 cm. and 20 cm. Fig. 13, transection of junction of storage region and heart of tuber; cambium-like layers lignified; heart without starch. Fig. 14, transection of cork, sclerenchyma, phellem and phellogen.

* *c*, cambium; *ck*, thin phellem; *emb*, embryo; *end*, endosperm; *f*, funiculus; *h*, "heart" of storage tuber; *i*, integument; *n*, nucellus; *p*, phloem; *pc*, pericarp; *pr*, parenchyma; *ph*, phellogen; *s*, starch; *sck*, sclerenchymatous phellem, hard cork; *st*, storage tuber; *t*, tylosis; *x*, xylem; x_1, protoxylem; x_2, secondary xylem.

latter constitutes the main bulk of the tuber. The largest bundles of the outer cells of the xylem lie adjacent to the vessels and are partially or completely



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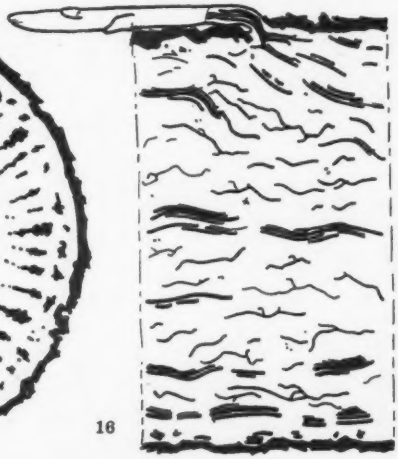

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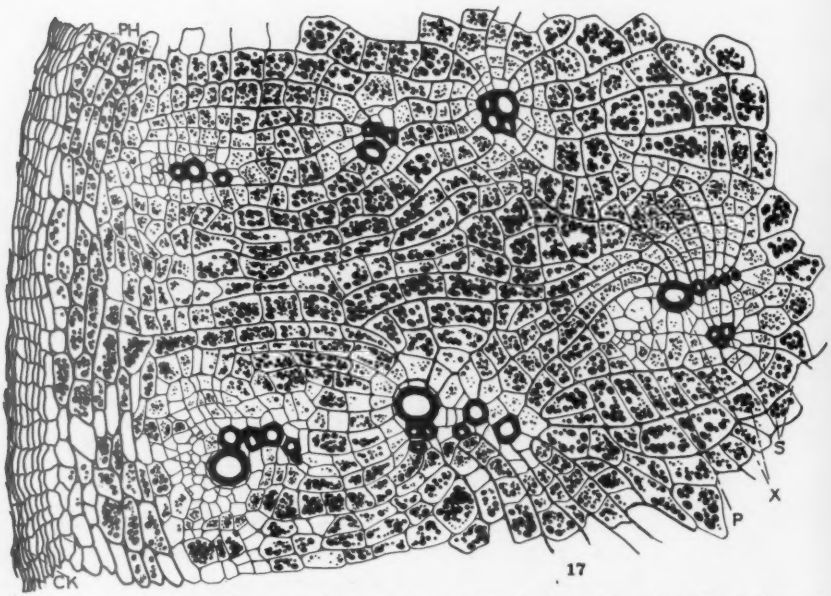

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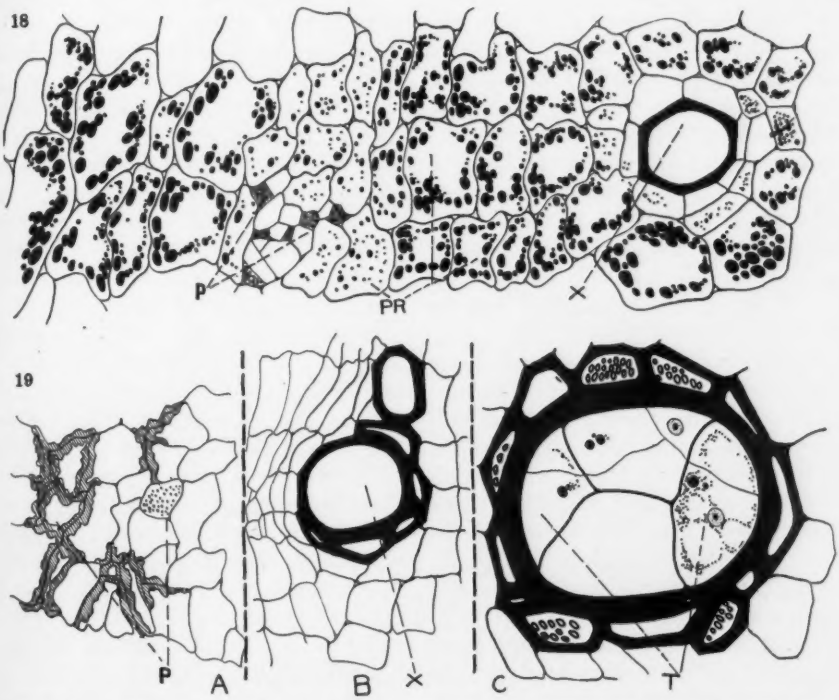
FIGS. 15–17.—Figs. 15, 16, diagrammatic transection and longisection of tuber; distribution of vascular strands. Fig. 17, transection of young tuber with vascular strands and starch distribution.

rows are collateral, with eight to twelve wide xylem vessels in roughly radial alignment. Tracheids and parenchyma lignified (figs. 17, 19). Smaller bundles of one to five vessels, with correspondingly limited phloem, alternate irregularly

with the larger ones. The conducting strands are deflected from the vertical in both tangential and radial directions. Anastomosis further complicates the pattern and results in a vascular network (fig. 16). In addition to the typical collateral bundles, isolated strands of xylem are transitory, and each successive cork cambium differentiates at a deeper level. As the cork develops, vascular strands and parenchyma in addition to old corky layers are sloughed off. Beneath the gnarled corky zone thus formed lie parenchyma and concentric rings of bundles



FIGS. 18, 19.—Fig. 18, transection of young phloem and xylem strands with intervening parenchyma. Fig. 19, *A*, *B*, *C*, transections of phloem, young xylem, and tylosed xylem strand in radial alignment; intervening parenchyma omitted.

and phloem are also evident. These may arise as independent units in the cambial region, separated at the time of origin by four to ten parenchymatous cells (fig. 18), or they may be forced apart later by secondary division of such cells. This division is readily detected by cell shape and relative wall thickness.

Older tubers are essentially similar in structure (figs. 12, 15, 16). Phellogens (fig. 14). Irregularities in certain sectors are dependent on the orientation and number of annual shoots. As in the younger tuber, vascular bundles and parenchyma are shed with the corky tissues. To attempt to estimate age in terms of seasonal rings is impossible. The central bundles are irregular in distribution, the result—as before—of secondary division of parenchymatous cells.

Xylem vessels are similar in structure throughout the tuber, except for the tyloses in the older elements (fig. 19*C*). Tylotic walls may show reticulate thickening. In transection the core of the tuber resembles heart wood in demarcation. It is amber in color and completely devoid of starch. Along three-eighths of its circumference, tuber and core are split apart, while the rest of the perimeter is marked by a cambium-like zone of cells, the walls of which are completely lignified. Further evidence of tissue contraction is seen in a central split within the heart wood itself (figs. 12, 13).

Seasonal shoots arise from meristem present in the narrow, irregular tuber neck. If the surface soil has been removed by erosion, chlorophyll may be present in the neck tissue.

Reserve material in the tuber includes starch, fat droplets, and protein material. Starch grains in cells adjacent to vascular strands are generally small, while those in the center of the rays (elliptical, spherical, or irregular in outline) may measure 40–65 μ in diameter (fig. 18). In young cells starch-containing leucoplasts usually encircle the nucleus, but later the grains are distributed throughout the cytoplasm. In the majority of the parenchymatous cells one or two fat droplets are present which stain brilliantly with Sudan III. In addition to these microscopically visible ergastic substances, colloidal protein is indicated by Millon's reagent. Phloem strands are particularly well defined. The paths of collateral vascular bundles and of independent xylem and phloem strands may be clearly compared and contrasted in two adjacent longitudinal slices of tuber, 3 mm. or less, treated with Millon's reagent or with phloroglucin and HCl.

RESERVE MATERIALS IN SHOOT.—As might be expected in short-lived tissues, the shoot contains no significant amounts of reserve at any time. Starch is evident in the chlorenchyma of the outer cortex; but in the inner cortex, rays, and pith it is negligible. Small granules of sieve-tube starch occur in the sieve tubes. Fat droplets, one or two in number, 1–4 μ in diameter, are usually present in parenchymatous cells. As in the tuber, protein is definitely and somewhat curiously localized. The deepest coloration appears in the sieve-tube contents, but cell walls also are heavily stained, in particular in collenchyma, pericycle, crushed phloem, and xylem. The actual protoplasts of the parenchyma, even after half an hour, remain only slightly stained. In tuber and in shoot, calcium oxalate is practically absent.

NUCLEAR SIZE IN SHOOT.—The nuclei of the isodiametric meristem cells are relatively large, 4 μ in diameter, 33 cu. μ in volume. Nuclear size is considered in reference to this basic volume, V = 33.[1]

In contrast to this, the giant nuclei of the differentiating vessel segments measure 20,000–300,000 cu. μ and therefore show as much as a 10,000-fold increase in volume. When immediate increase is considered, the reference point is necessarily the nucleus of a cambial cell, 432 cu. μ, or 13V. The direct growth from cambial nucleus to vessel nucleus is thus 763-fold only (table 1).

The parenchymatous elements of the tuber cortex, rays, and pith may approach the vessel segments in diameter and equal or exceed them in length. The nuclei are consistently smaller, however, with a maximum of 180,757 cu. μ (5,477V, in contrast to the previous figures).

Measurement in chlorenchymatous cells is practically impossible, since chloroplasts consistently surround the living

[1] Throughout this paper, V is used for volume instead of the letter X of the original papers.

nucleus. The largest nucleus noted, 6,186 cu. μ, indicates 187-fold volume increase. The volume frequencies for nuclei of parenchyma and vessel segments are summarized in table 2. Maximal frequencies for the larger xylem elements occur at 12,095, 28,670, 55,992, and 96,762, and at 163,643 a lesser peak. This

TABLE 1

RANGE OF NUCLEAR SIZE IN SHOOT OF ECHINOCYSTIS MACROCARPA IN: *A*, APEX AND INTERNODES 2–13 APPROXIMATELY; *B*, REGION OF EXPANSION AND OF DIFFERENTIATION OF SPIRAL VESSELS, ETC.; INTERNODES 14–26 APPROXIMATELY; *C*, REGION OF ACTIVE SECONDARY THICKENING; INTERNODES 27–30 APPROXIMATELY; *D*, MATURE REGION, OFTEN WITHOUT LEAVES, TENDRILS, OR BUDS IN LOWEST INTERNODES

Region of stem	Cell		Nucleus		Nucleolus diameter (μ)	Scale of increase
	Type	Dimensions (μ)	Diameter (μ)	Volume (cu. μ)		
A	Meristematic, dividing	8 (isodiametric)	4	33	2	33=V
	Vacuolating, dividing	13–30 (isodiametric)	5–9	81–432	2–3	2.4–13V
B	Epidermis	22×13–22×22	8	267	3	8V
	Trichomes	19×40–20×13	6	163	2	4V
	Glandular hairs "head"	12=radius of sphere quadrant	8	267	2	8V
	Collenchyma	95×17–152×28	11	771	3	23V
	Chlorenchyma	66×38	22	6,186	5	187V
	Parenchyma of cortex, rays, pith	70×51–123×62	17–38	2,935–28,670	3–6	88–868V
	Spiral vessels	32–38 (diameter)	17–22	2,935–6,186	3–4	88–187V
C	Epidermis	150×20	9	432	3	13V
	Collenchyma	209×22–228×26	9–11	432–771	3–4	13–23V
	Chlorenchyma	47×38–76×38	19	3,582	5	108V
	Pericyclic fibers	200×25–700×30	9–11	432–771	3–5	13–23V
	Parenchyma of cortex, pith, rays, xylem, etc.	95×66–475×114	11–38	771–28,670	3–5	23–859V
	Cambium	47×17–47×22 (transection)	5–9	91–432	2–3	2.7–13V
	Vessel segments	49–437 (diameter)	9–85	432–330,023	3–12	13–10,000V
D	Epidermis	114×15–228×28	11	771	3	23V
	Collenchyma	209×30	11	771	3	23V
	Chlorenchyma	47×34–250×32	9–11	432–771	3–3	13–23V
	Pericyclic fibers	456×32–760×32	13–19	1,200–3,582	2–4	36–108V
	Parenchyma of cortex, pith, rays, xylem, etc.	209×30–320×160	11–41	771–38,159	2–6	23–1,156V
	Cambium	28×11 (transection)	9	432	3	13V
	Vessel segments	96–285 (diameter)	28–70	12,095–180,757	3–22×19	366–5,477V

The remaining tissues include trichomes and epidermal cells which range from 4V to 23V, collenchyma elements 13V to 23V, and pericyclic fibers 13V to 108V.

series falls approximately into the V, 2V, 4V, 8V series of MONSCHAU (6), the significance of which is discussed later.

CYTOLOGY OF GIANT NUCLEI.—The giant nuclei of the vessel segments and of the parenchyma appear to be in the resting or early prophase condition and

TABLE 2

VOLUME FREQUENCIES OF NUCLEI IN XYLEM AND IN PARENCHYMA OF ECHINOCYSTIS MACROCARPA

VOLUME (CU. μ)	FREQUENCY		VOLUME (CU. μ)	FREQUENCY	
	Xylem	Parenchyma		Xylem	Parenchyma
91...	.....	3	24,578...	1	.....
229...	.....	14	28,670...	48	10
432...	.....	27	33,327...	8	1
771...	4	36	33,433...	2	.....
1,200...	4	36	38,159...	15	.....
1,832...	10	22	43,601...	13	5
2,935...	10	15	44,600...	1	.....
3,582...	7	23	49,541...	1	.....
4,773...	10	11	55,992...	64	3
6,186...	18	17	62,980...	3	1
7,890...	21	9	70,521...	9	.....
9,605...	3	.....	79,081...	8	.....
9,827...	7	.....	87,403...	1	.....
12,095...	34	14	96,762...	34	.....
12,866...	2	.....	115,989...	1	.....
14,081...	7	1	117,424...	6	.....
15,033...	1	.....	117,731...	5	.....
15,479...	2	.....	128,164...	3	.....
17,607...	17	4	163,643...	12	.....
18,599...	3	.....	180,757...	2	1
19,345...	1	.....	229,364...	3	.....
20,900...	18	4	330,023...	2	.....
22,935...	3	.....			

are essentially similar to those in *Ricinus* (9). A reticulum (fine or coarse) is visible in untreated nuclei and is accentuated but not altered in outline by Feulgen's reagent. The nucleoli are variable in number, size, and shape. The nucleolar material is heterogeneous, with minute refractive droplets, 0.5 μ or less in diameter, scattered irregularly throughout (fig. 20*A*–*E*).

Cytoplasmic streaming along cell walls and in radiating protoplasmic strands indicates that the living cells are not killed in the process of sectioning. Nuclei when first detected are practically invisible, since they are translucent and faint in outline. Change in refractive index is more or less immediate, and the nucleus becomes microscopically clear. That increased visibility may actually entail decrease in nuclear volume, perhaps as a result of the shock of sectioning, is evident in parenchymatous cells. In longitudinal section the cells in any one vertical file are equal in diameter and approximately equal in length. In cells where protoplasmic streaming is apparent, the nucleus is translucent and is generally outlined by a sheath of chloroplasts. In the cells where protoplasmic streaming can no longer be detected, the nucleus is perfectly visible, and the halo of chloroplasts, perhaps slightly disturbed, now lies more or less removed from the nuclear surface. Table 3 shows that the diameters of living nuclei measure approximately twice those of nonliving.

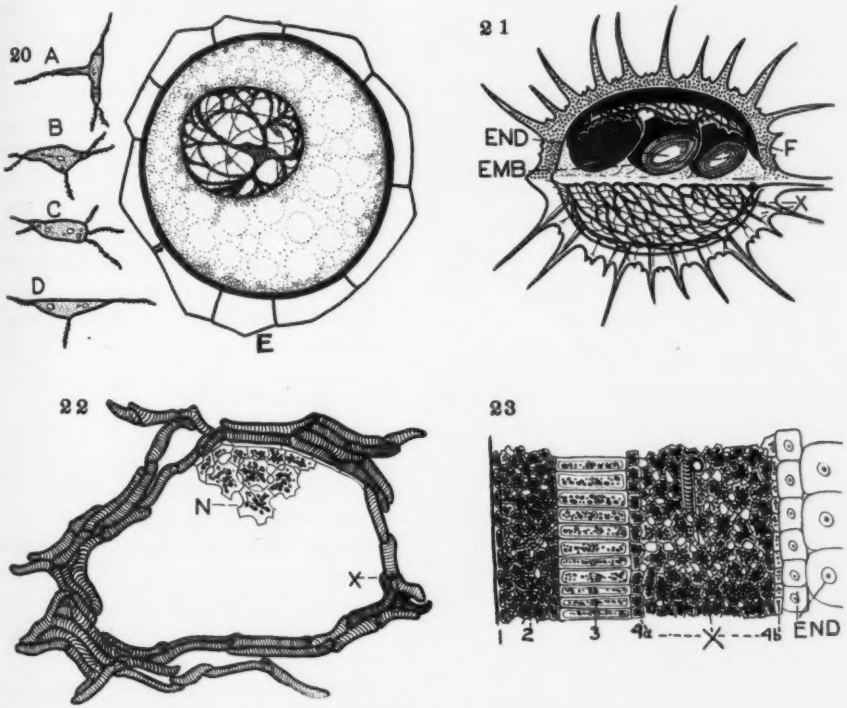
Re-examination of earlier figures indicates that the variability in nuclear size in parenchymatous cells of approximately the same dimensions is doubtless due to this contrast between living and nonliving units.

Extreme nuclear contraction may be induced, as in *Ricinus*, by dehydration with absolute alcohol (8). When fresh sections are irrigated with alcohol, nuclear shrinkage begins immediately on penetration of the reagent and continues until equilibrium is reached. This minimum volume remains unaltered by addition of xylene. The percentage decrease in volume may be taken to indicate the percentage volume of "free" water. As the abbreviated figures in table 4 show, the amount varies from 39 to 82 per cent. The contracted nuclei are still much larg-

er than those of the apical meristem. The absolute increase in nuclear material estimated by comparison with the latter ranges from 86 to 232V.

FRUIT AND SEED.—The pendent fruit, a capsule, is spherical to ellipsoidal in shape, densely spiny, and measures 6–8 cm. in length (fig. 21). The carpels range from two to six in number, and the placentation is axile (4). The seeds are ellipsoidal, about 1 cm. long, and vary from one to four per carpel. They are shed while the fruit is still green. The latter breaks open by ragged irregular tears around the beak and thereafter dries out and becomes brown and papery.

The surface of the young ovary is densely covered with trichomes and glandular hairs. Typical epidermal cells are approximately isodiametric, and stomata are frequent. The glandular hairs generally persist, more or less dried up, in the older fruits and may be identified even at the spine tips. The mesocarp of the young fruit is spongy and consists of chlorenchyma and parenchyma. Intercellular spaces appear early in the latter and indicate the future zones of tissue shrinkage and splitting which accompany locule development. The vascular network is highly complex. The cells of the endocarp are small and tabular and



FIGS. 20–23.—Fig. 20, *A–D*, varying outlines of nuclei; *E*, giant nucleus in differentiating vessel segment. Fig. 21, diagrammatic longisection of semimature green fruit with developing ovules, venation of carpel, and funiculus. Fig. 22, vascular network of nucellus. Fig. 23, semidiagrammatic transection of seedcoat of semimature seed: epidermis (*1*), parenchyma (*2*), palisade layer (*3*), nucellus with outer and inner limiting layers (*4a*, *4b*), and spiral vessels of vascular network.

generally adhere to the surface of the ripening seed.

The seed, when mature, is light brown or buff in color; the embryo is typically dicotyledonous. While unripe it is white,

TABLE 3

COMPARISON OF NUCLEAR DIAMETERS AND VOLUMES IN CLOSELY ADJACENT PARENCHYMA CELLS, LIVING AND NONLIVING, OF ECHINOCYSTIS MACROCARPA

REGION OF STEM	No.	DIMENSION OF CELL (LONGISECTION)	NUCLEUS		DIAMETER OF NUCLEOLUS	APPEARANCE OF NUCLEUS
			Diameter (μ)	Volume (cu. μ)		
C	1	475×114	38	28,670	5	S.T.*
	2	456×114	19	3,582	3	R.O.†
C	1	133× 66	24	7,890	5	S.T.
	2	98× 66	20	4,773	4	S.T.
	3	95× 66	11	771	3	R.O.
D	1	400× 72	32	17,607	5	S.T.
	2	440×120	17	2,935	4	R.O.

* Nucleus closely surrounded by chloroplasts and translucent.

† Chloroplasts removed from nuclear surface, nucleus opaque and either granular or recticular.

TABLE 4

RANGE OF CONTRACTION OF NUCLEI OF ECHINOCYSTIS MACROCARPA ON ADDITION OF ABSOLUTE ALCOHOL; VESSEL DIAMETER 100–200μ

Nuclear volume (cu. μ)	Volume after shrinkage (cu. μ)	Water loss (%)	Comparison with cambial volume (V=91 cu. μ)
28,670	7,890	72	86V
35,024	21,125	39	232V
96,762	16,385	82	185V

with a distinct ridge outlining the major ellipse. If the seed at this stage is removed from the capsule and exposed to the air, the tannin in the cells of the outer seedcoat immediately darkens to olive brown or gray, and the ridge stands out as a darker line. In such a semimature seedcoat four layers may be distinguished:

1. The epidermal cells, in general isodiametric, are thickened on the external walls and are already suberized. Starch and tannin are present throughout (fig. 23 *1*).
2. The underlying parenchymatous elements are loosely packed, isodiametric to oblong, with abundant starch and some tannin. In the mature seed the walls thicken and lignify, ending as a layer of fibrous sclerenchyma (fig. 23 *2*).
3. Palisade cells, 600–700 μ in length and about 60 μ in diameter, form the main strengthening layer of the mature seedcoat. At this early stage wall thickening is just beginning along the outer tangential and radial walls. Starch grains are rather numerous. Suberization, lignification, and further thickening of walls occur later (fig. 23 *3*).
4. The nucellus, bounded on the outer and inner surfaces by more or less tabular cells, consists of loose spongy tissue supported by a vascular network. The much-branched spongy cells are packed with starch, and intercellular spaces are definite. The lignified spiral elements of the vascular mesh are accompanied by a narrow line of phloem-parenchyma. The cells of the limiting layers are tabular and contain some starch (fig. 23 *4a*, *4b*, *X*; fig. 22).

The endosperm is clear and glassy and practically devoid of starch. In the larger central cells the nuclei are readily visible with a hand lens (fig. 23 *END*). The endosperm is partially surrounded by the cotyledons of the developing embryo. The cotyledonary cells are relatively small and packed with protein granules 1–2 μ in diameter. Addition of Millon's reagent results in the immediate deep-flesh coloration seen in the tuber paren-

chyma. A few small starch grains are detected in most cells on addition of IKI.

As the seed matures, the reserve starch disappears from integument and nucellus. The nucellus itself is crushed and more or less completely resorbed as the embryo replaces the endosperm. The seedcoat as a whole hardens with suberization and lignification of the epidermis and of the underlying parenchyma and palisade layers.

NUCLEAR SIZE IN FRUIT AND SEED.—The nuclei of the pericarp in general resemble those of the vegetative tissues in size range. Endosperm nuclei, at a certain stage of development, are conspicuously larger but do not equal the maximum volumes of the vessel segment nuclei. Table 5 gives typical figures.

TABLE 5

NUCLEAR SIZE IN FRUIT AND SEED TISSUES OF ECHINOCYSTIS MACROCARPA

Region	Tissue	Cell dimensions (μ)	Diameter		Volume of nucleus (cu. μ)
			Nucleus (μ)	Nucleolus (μ)	
Fruit	Epidermal hairs	24×24	9	3	432
		114×24	11	3	771
	Chlorenchyma outer mesocarp	76×76	20	4	4,773
		171×85	19	3	3,582
	Parenchyma inner mesocarp	228×228	28	5	12,095
		322×190	38	5	28,670
Seed	Palisade cells	648×60	28	6	12,095
	Endosperm	228×228	41	7	33,327
		182×182	49	4	62,890
		436×228	57	3	96,762
		228×228	62	4	128,164

B. CUCURBITA PEPO

The axis of *C. pepo*, reviewed fully by HAYWARD (4), is similar to that of *Echinocystis*. The spiral vessels of the primary bundles are followed by large pitted vessels right and left of the original xylem wedge. Later secondary vessels differentiate between the pitted laterals. The region of most active secondary growth, the seventeenth to nineteenth internodes, inclusive, in the specimens examined is the region most useful in the study of nuclear size (tables 6, 7, 8). The maximum volumes of nuclei in xylem and in parenchymatous cells, 4,958V and 1,156V, when reckoned in comparison with the nuclei of the meristem cells (V = 33 cu. μ), do not equal the largest

TABLE 6

NUMBER OF DIFFERENTIATING VESSELS, MAXIMUM VESSEL DIAMETERS, MAXIMUM NUCLEAR DIAMETERS, AND VOLUMES IN 40-CM. SHOOT OF CUCURBITA PEPO IN SUCCESSIVE INTERNODES

Region*	Internode number	Vessels			Nucleus	
		Number differentiating		Diameter of largest lignified vessel (μ)	Diameter (μ)	Volume (cu. μ)
		Inner bundles	Outer bundles			
B	14†	34	39	66	28	12,095
	15	25	25	83	51	70,521
	16	12	3	142	38	28,670
C	17	8	4	237	49	62,980
	18	6	7	218	57	96,762
	19	3	8	180	43	43,601
	20	5	6	190	41	38,159
	21	5	6	199	41	38,159
D	22	0	2	171	47	55,992
	23	3	4	218	38	28,670

* Apical region (A), one to thirteen internodes.
† Approximately.

nuclei seen in *Echinocystis*. It is possible that this is due to the fact that the *Cucurbita* seeds were sown rather late in summer, and growth was not so vigorous as in the early part of the season.

TABLE 7

RANGE OF NUCLEAR SIZE IN 215-CM. SHOOT OF CUCURBITA PEPO

Region	Cell		Nucleus		Nucleolus diameter (μ)	Scale of increase
	Type	Diameter (μ)	Diameter (μ)	Volume (cu. μ)		
A	Meristematic, dividing	7– 8	4	33	2	V=33
B	Vessels	36– 85	13–51	1,200– 70,521	3–6	38–140V
C	Parenchyma	123–266	15–41	1,832– 38,159	3–6	55–1,156V
	Vessels	152–266	30–66	14,801–163,643	3–6	478–4,958V
D	Vessels	60–171	22–49	6,186– 62,980	3–6	187–1,908V

TABLE 8

VOLUME FREQUENCIES IN PARENCHYMA AND VESSELS OF CUCURBITA PEPO

Volume (cu. μ+)	Parenchyma	Vessels	Volume (cu. μ+)	Parenchyma	Vessels
1,000	3	1	28,000	.....	32
3,000	3	4	33,000	.....	13
5,000	2	6	43,000	.....	2
7,000	2	10	55,000	.....	8
9,000	2	.....	62,000	.....	14
11,000	1	5	70,000	.....	8
13,000	1	12	79,000	.....	1
17,000	1	10	96,000	.....	2
19,000	.....	12	163,000	.....	1
24,000	.....	2			

Discussion

While apparently only one paper in the literature deals with nuclear size and vessel differentiation (**9**), considerable data has accumulated around the problem of nucleocytoplasmic ratios in general. Among other papers (**11**), the recent studies of the changing rates of growth in cells and in nuclei by SINNOTT and TROMBETTA (**10**) provide the most illuminating method of attack and graphic expression of the problem. Isolated facts observed in the present survey, if subjected to such statistical study and critical analysis, could be fitted into their place in the cycle of growth. For example: (*a*) The nuclei of very young differentiating vessel segments may measure approximately seven-eighths of the vessel diameter, indicating that the nucleus in the early stages of vessel growth increases much more rapidly than the protoplast as a whole. The point at which nuclear growth begins to slacken remains undetermined. (*b*) Nuclei in adjacent parenchymatous cells of exactly similar diameter and length may differ greatly in volume. This may be due to sectioning shock or to cell age. (*c*) In contrast to this, nuclei of like volume may appear in adjacent cells of equal diameter but of markedly different length and therefore volume. Since the size of nuclei from the apical meristem downward increases with increasing cell size (table 1), this uniformity requires explanation. Cell age may or may not be the reason.

The ratio of volume of cell/volume of nucleus is highly variable in *Echinocystis*, as in other plants (**2, 10, 11**). Calculations are valid only when based on detailed measurements. The following figures merely illustrate the range seen in

typical cells in various tissues: (*a*) meristem 15:1; (*b*) cortical parenchyma 12:1–113:1; (*c*) differentiating spiral vessel 18:1; (*d*) differentiating pitted vessels 34:1–152:1.

The volume frequencies in table 2 recall the serial figures of nuclear doubling of JACOBY (**5**) and MONSCHAU (**6**), V, 2V, 4V, 8V, which are refuted by WERMEL and PORTUGALOW (**12**). It is expected that a more complete statistical analysis of *Echinocystis* and also of *Ricinus* (**9**) would result in a uniform curve instead of a many-peaked graph. The theory of protomere division is not necessarily ruled out, but time intervals between individual divisions throughout the mass doubtless vary with internal and external milieu. As a result the stages in nuclear growth will probably present a gradual and continuous sequence.

More recent cytological work indicates that nuclear volume must be considered in relation to chromosome number (**3**). Whether the vessels and parenchymatous cells of *Echinocystis* and *Cucurbita* are actually polyploid is undetermined.

Nuclei as osmometers are discussed by CHURNEY in isolated *Arbacia* and other eggs (**1**). Certain tentative experiments were made with sections of *Echinocystis*, but the contents of the nuclei responsible for the increasing intake of water remain to be determined.

Summary

1. In *Echinocystis macrocarpa* the large differentiating vessels of the secondary xylem furnish excellent material for the study of giant nuclei.

2. The massive tuber is made up of storage parenchyma and a complex vascular network. Microscopically visible ergastic substances include abundant starch and a few small oil droplets. Immediate reaction with Millon's reagent indicates high protein. The core of the tuber is demarcated by amber color, lack of starch, and tissue shrinkage resulting in cleavage cracks.

3. Ergastic substances in the short-lived shoot are limited to rather abundant starch in the chlorenchyma of the outer cortex and in the sieve tubes and scattered starch grains and oil droplets in other tissues. High protein is indicated in sieve tubes and curiously also in the cell walls of collenchyma, pericycle, crushed phloem, and the xylem. Calcium oxalate is absent throughout practically the entire plant.

4. Structure and development of fruit and seed are outlined. Reaction is marked in the microscopically visible granular protein of the embryo cotyledons.

5. The differentiating vessels contain nuclei of relatively great size, the maximum 85 μ in diameter and 330,023 cu. μ in volume. This confirms the opinion expressed after the study of *Ricinus* that giant nuclei are of general occurrence in wide-vesseled plants.

6. The scale of nuclear growth is reckoned in comparison with the size of the meristem nuclei, 33 cu. μ = V. The cambial nuclei remain consistently small, 432 cu. μ = 13V, while the nuclei of hairs, collenchyma, and other vegetative tissues range from 4 to 187V. Endosperm nuclei measure 3,883V during early stages of growth.

7. Giant nuclei appear to be either in the resting or in the prophase condition. A surface network of chromatin is apparent. The nucleoli, one or more in number, are extremely variable in outline and contain minute, highly refractive droplets.

8. Shrinkage in volume, probably due to water loss, may result from sectioning. Nuclei in living parenchymatous cells

when first mounted are outlined by encircling chloroplasts, translucent and therefore practically invisible. Their diameter is approximately twice that of nuclei in exactly similar closely adjacent nonliving cells. (A nonliving cell is here defined as one in which no trace of protoplasmic rotation or circulation can be detected.)

9. Contraction in volume is induced by dehydration of fresh material with absolute alcohol. The volume of free water ranges from 39 to 82 per cent. Actual increase in nuclear material is reckoned by comparison of the contracted nuclei with the meristem nuclear volume, V = 33, and amounts to 86–232V.

10. The ratio of cell volume/nuclear volume in isolated typical cells from various tissues illustrates the variability of this figure (15:1 in meristem to 152:1 in vessels).

11. *Cucurbita pepo* is essentially similar to *Echinocystis* in the anatomy of the shoot. The nuclei in the developing vessels are somewhat smaller and reach a maximum of 66 μ and 163,643 cu. μ in diameter and volume.

12. Comparison of living material and prepared slides on both *Echinocystis* and *Cucurbita* indicates the necessity of the former in all studies pertaining to cell and nuclear growth.

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TRANSLOCATION OF THE FLORAL STIMULUS IN XANTHIUM[1]

ALICE P. WITHROW AND R. B. WITHROW

(WITH THREE FIGURES)

Introduction

HAMNER and BONNER (4) have shown that in a two-branched plant of *Xanthium*, if one branch (designated the donor branch) is induced to initiate flower buds by a short photoperiod treatment, the floral stimulus is translocated to the receptor branch kept on a long photoperiod and the receptor branch flowers. They have further shown that the stimulus will cross an inarch graft union under similar conditions with the same result. They also found that the receptor plant flowered if a membrane of lens paper was placed between the cut surfaces of the graft stems and concluded that the stimulus passed through a nonliving diffusion contact, and therefore that a definite substance was involved in causing flowering. On the other hand, MOSHKOV (5) concluded from his experiments with *Perilla* that tissue union between the scion and stock was necessary for the transfer of the floral stimulus.

Little work has been done to show through what tissues of the stem the floral stimulus travels from its point of origin to the induced bud. CAJLACHJAN (2) has reported that the stimulus in *Perilla* was translocated both up and down the stem in the tissues of the bark.

The present experiments were designed to study the translocation of the floral stimulus in *Xanthium pennsylvanicum* in relation to its movement through diffusion contacts and to the tissues involved in its translocation in the petiole and stem.

[1] Journal Paper no. 53 of the Purdue University Agricultural Experiment Station.

Investigation

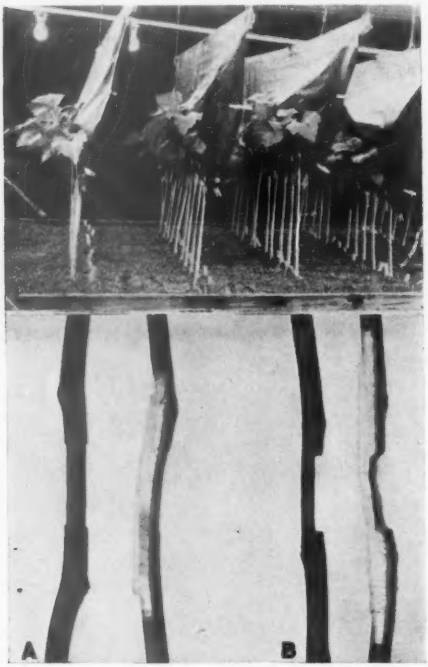
INARCH GRAFTS AND DIFFUSION CONTACTS

It appeared reasonable to assume that if the floral stimulus from a donor plant could cross a diffusion contact to a receptor plant, something of its nature could be found if various types of diffusion membranes were used. This would aid materially in selecting the most suitable solvent for extraction purposes.

Preliminary experiments using inarch graft contacts through various membranes were set up. Thin cellophane, thin rice paper (cigarette paper), and thin rice paper impregnated with various substances were used as membranes. The impregnating agents included lanolin, a polybutene–mineral oil mixture, and beeswax. It was found that the floral stimulus did not cross any of these contacts, and only those plants flowered where a direct graft was made and tissue union allowed to take place.

Following the preliminary experiments, it was decided to limit the types of membranes used and to incorporate experiments in which tissue union of direct type grafts was restricted. The following types of inarch contacts were made: (*a*) direct graft which was left undisturbed; (*b*) direct graft taken apart every 4 days, with the stem surfaces recut the first four times; (*c*) direct graft taken apart every 8 days, with new surfaces cut the first two times; (*d*) a lens-paper membrane interposed; (*e*) a thin rice-paper membrane interposed; (*f*) a lanolin-impregnated rice-paper membrane interposed.

In one case the donor plants of the pair were induced with short 10-hour photoperiods and allowed to come into flower before the contacts were made, at which time the short photoperiods were discontinued and the pairs of inarched plants placed on a 24-hour photoperiod



FIGS. 1, 2.—Fig. 1 (above), method used in curtaining donor plants. Fig. 2 (below), stem of donor branch: *A*, from which ring of bark has been removed, before and after taping; *B*, from which half-section has been removed, before and after taping.

(continuous irradiation) of solar irradiation supplemented with 20 footcandles of incandescent lamp irradiation. In these and subsequent experiments the receptor plants were defoliated to two well-developed leaves at the time of grafting and the burs were removed from the donor plants at intervals throughout the test period. This experiment was conducted during April and May.

In the second case induction of the donor was begun at the time the grafts were made, and the donor plant was curtained with heavy rubberized black cloth throughout the experimental period so as to receive 10 hours of solar radiant energy daily, while the receptor plants were subjected to the 24-hour photoperiod just indicated. This work was done during June and July. Figure 1 shows the grafted plants with curtains in place. All contacts were of the inarch type, about 20 cm. in length, and were securely bound with Parafilm tape. Rubber bands which had been cut into single lengths were wrapped around the outside of the Parafilm tape. This procedure kept the two stems in close contact but allowed adequate opportunity for stem growth without girdling.

The plants were grown in pots in sub-irrigation gravel culture beds with a complete nutrient solution. At the end of the experimental period of 50 days, freehand transections of the contacts were made in most cases to determine whether tissue adhesion or actual tissue union obtained. In all cases the grafts and contacts were subsequently taken apart and the condition of the membranes and state of tissue union noted. Dissection under a low-power binocular microscope was made for flower buds in all the experiments.

Table 1 summarizes the results. No receptor plants flowered except those which had formed tissue union with the donor plants. The flowering was restricted largely to those plants with direct undisturbed grafts and those with lens-paper membranes. Tissue union seldom failed to occur under both conditions, although frequently the areas of union were very small when lens paper was interposed between the cut surfaces.

Of thirty-three receptor plants where the rice-paper membranes were used, only one flowered, and it was found that the paper had disintegrated early enough to allow tissue union to take place. A similar situation existed where one receptor plant with a lanolin–rice-paper membrane flowered.

When the donor plants were induced previous to grafting and the short photoperiods over the donor discontinued at the time of grafting, less than half the plants in the treatments with direct graft and lens-paper membrane flowered, even though tissue union occurred. When the induction was begun at the time of grafting, 90 per cent or more of the plants flowered. When the direct contact was disturbed every 4 days, no flowering occurred although tissue adhesion had taken place at the end of 4 days. When the direct grafts were taken apart every 8 days, some of the plants eventually flowered, although the flowering was delayed as compared with undisturbed direct grafts. With freehand transections of test plants, tissue union was observed in about 60 per cent of them when the grafts were left in direct contact for 8 days. It is entirely possible that union occurred on the remainder of the plants at some point on the graft not observed with the microscope. Strong adhesion occurred in all cases.

The plants with the lens-paper membranes developed burs more rapidly than did those with direct grafts. This was observed in both experiments.

TABLE 1

FLOWERING RESPONSE OF RECEPTOR PLANTS OF XANTHIUM

Treatment	I. Donors induced previous to grafting	II. Donors induced after grafting
	Receptors with flower buds 39 days after grafting* (%)	Receptors with flower buds 28 days after grafting* (%)
Direct graft	39	93
Lens-paper membrane	39	100
Rice-paper membrane	0	7†
Lanolin-impregnated rice-paper membrane	0	7†
Direct contact disturbed every 4 days	0	0
Direct contact disturbed every 8 days	0	20

* No change in flowering response at 50 days; 18 pairs of plants per treatment in I and 15 pairs in II.

† Tissue union between the donor and receptor plants occurred where flowering took place.

Translocation across dead petiole tissue

Since it appeared that tissue union was necessary for transfer of the floral stimulus from one plant to another, it was thought desirable to determine whether the stimulus could be translocated from the induced leaf of a plant across a small length of dead petiole tissue.

The plants, growing in subirrigation pot culture with complete nutrient solution, were defoliated to two young mature leaves and the younger developing leaves. One mature leaf was left intact and uninduced; the second received the following treatments, 25 plants per treatment: (*a*) uninduced on long photoperiod; (*b*) induced on short photoperiod with intact petiole; and (*c*) induced on short photoperiod with petiole section approximately 1 cm. in length killed by steaming for 15 seconds.

The petiole to be treated with steam was incased in two pieces of slit rubber tubing which were separated so as to expose 1 cm. of the petiole to the steam. The petiole was then clamped between two blocks of wood for further protection. Steam was applied by means of a

small glass jet inserted into a 1-cm. hole drilled in the wood blocks. Immediately after steaming, the petiole was bound

FIG. 3.—Leaf 24 hours after petiole section was killed by steaming for 15 seconds.

TABLE 2

EFFECT OF KILLING PETIOLE SECTION OF INDUCED LEAF ON FLOWERING RESPONSE OF XANTHIUM

Treatment	Plants with flower buds at 31 days* (%)
Short photoperiod, petiole intact....	84
Short photoperiod, petiole steamed 15 seconds.....	0
Long photoperiod, petiole intact....	0

* No change in flowering response at 40 days; 25 plants per treatment.

with thin wood splints and Parafilm tape to keep it in position. Figure 3 shows a leaf on the morning following the steaming treatment. The steamed petiole sections were greatly shrunken and completely brown at this time.

All the plants were kept on a 24-hour photoperiod of solar radiant energy supplemented by 20 footcandles of incandescent lamp irradiation. The leaves to be induced were placed in heavy black-paper envelopes each day at the end of 10 hours of solar irradiation. These leaves were removed from all the plants after 4 days of induction. At this time all the leaves appeared turgid and healthy, including those with steamed petioles. Preliminary experiments had shown that considerable wilting of the leaves with steamed petioles took place 5 days after treatment. The results are given in table 2. Only those plants flowered which had induced leaves with intact petioles.

TRANSLOCATION OF FLORAL STIMULUS DOWN STEM

Since it appeared from the previous experiments that the floral stimulus failed to diffuse across a nonliving membrane and that it was not translocated down through a section of dead petiole tissue whose xylem vessels were functional, it was thought likely that the stimulus was translocated within the plant through the living cells of the stem. An experiment was conducted to determine whether the downward movement of the stimulus was restricted to the bark.

The apical bud was pinched from a group of plants and two equal-sized branches allowed to develop. When these were large and well developed, the plants were divided into three groups. In all cases, one (donor) branch of each plant was curtained with the black cloth so as to receive a 10-hour photoperiod. The second (receptor) branch received a 24-hour photoperiod of solar radiant energy supplemented by 20 footcandles of incandescent lamp irradiation. In one

group the plants were left intact as controls; in the second group a ring of bark 4 cm. long, completely girdling the stem, was removed from the donor branch about 20 cm. above the point of branching. In the third group a half-section of the stem about 4 cm. in length was removed from the donor branch at a similar point to check the possibility of the influence of tissue injury or reduction in amount of stem tissue on the translocation of the floral stimulus. On fifteen plants in each treatment the donor branch was removed at the end of 12 days' induction and on twenty plants at the end of 8 days. These two experiments were conducted at different times. Microscopic examination of freehand stem sections of plants grown in preliminary experiments showed that considerable regeneration of tissue occurred 8–16 days after tissue removal, depending upon growth conditions, and that formation of vascular elements occurred within a slightly longer period. Microscopic examination of freehand transections of the girdled stem portion was made at intervals on test plants during the experimental period, and transections of the center area of all the girdled stem portions were examined at the time the donor was removed. In this area no regeneration of tissue was found on many plants, while on others there were occasional small groups of newly formed parenchyma cells present external to the xylem. Whether these groups were continuous throughout the length of the girdle was not determined.

Figure 2 shows the stems after tissue removal before and after taping. Large branches were most suitable, since their bark could be removed without scraping. In small young plants the bark does not peel off readily.

The lower leaves of both branches were removed in all cases at the beginning of the induction period, and the induced leaves on the donor branch were above the point of tissue removal. The plants were grown in subirrigation gravel culture with complete nutrient solution.

The results are given in table 3. The receptor branches showed macroscopic floral buds as early as 15 days after induction of the donor branch was begun.

TABLE 3

EFFECT OF REMOVAL OF STEM TISSUES OF DONOR BRANCH ON FLOWERING OF RECEPTOR BRANCH IN TWO-BRANCHED PLANTS OF XANTHIUM

Treatment	I. Receptor branches with flower buds at 25 days* (%)	II. Receptor branches with flower buds at 32 days* (%)
Donor branch intact	100	.....
Donor branch with half-section removed	73	80
Donor branch with bark removed	0	5

* No change in flowering response at 45 days; 15 plants per treatment in I with donor branch removed after 12 days' induction and 20 plants per treatment in II with donor branch removed after 8 days' induction. No intact plants included in II.

However, the number of days given in the table is that after which no change occurred in the number of flowering receptor branches.

TRANSLOCATION UP STEM

Two-branched plants were divided into three groups, one branch serving as donor and given short photoperiods and the second serving as receptor and kept on long photoperiods. In these experiments it was the donor branch which was left intact. In group I the receptor branches were also left intact. In group II a complete section of bark 4 cm. long, entirely girdling the stem, was removed from the receptor branch 20 cm. above the point of branching. In group III a half-section 4 cm. in length was removed

from the stem of the receptor branch 20 cm. above the point of branching. Immediately after tissue removal, the treated stem portions were bound with Parafilm tape and supported with thin wood splints taped on to the stem. Just above the point of tissue removal the stem was wrapped with moistened sphagnum moss to facilitate the development of roots. This was done both where bark and half-stem sections were removed. In preliminary experiments those portions of the plant above the point of bark removal developed severe mineral deficiencies in 2–3 weeks, since the translocation of mineral elements was restricted. Such receptor branches could not be kept alive long enough to secure complete flowering data unless provision was made for a supply of mineral nutrients through a new root system developed above the girdle.

TABLE 4

EFFECT OF REMOVAL OF STEM TISSUES OF RECEPTOR BRANCH ON ITS FLOWERING IN TWO-BRANCHED PLANTS OF XANTHIUM

Treatment	Receptor branches with flower buds at 23 days* (%)
Receptor branch intact	100
Receptor branch with half-section removed	80
Receptor branch with bark removed	0

* No change in flowering response at 45 days; 20 plants per treatment with receptor branch removed after 14 days.

Immediately after tissue removal the donor branches were given short photoperiod treatment, which was continued for 14 days. At the end of this time the receptor branches, which now had well-developed roots, were removed from the plant immediately above the point of tissue removal and planted in subirrigation gravel culture with complete nutrient solution.

The results are given in table 4. The plants which were left intact all flowered, and 80 per cent of those with the half-section removed from the receptor branch flowered. Those with a complete ring of bark removed from the receptor branch failed to form floral primordia.

Discussion

The failure of the floral stimulus to cross the membranes used does not mean that there was no diffusion through them, but it does mean that the diffusion was not in sufficient quantity to cause flowering. Callus formation on the cut surfaces also may have decreased the opportunity for passage. These limitations, however, do not apply to the series disturbed every 4 days. Tissue adhesion occurred by the end of each 4-day period for the first 16 days, although no signs of union were observed when microscopic examination was made on test 4-day grafts run simultaneously. That the flower-forming stimulus failed to cross this tissue-to-tissue contact indicated that, under the conditions of the experiments, it was necessary for union to take place before translocation from the donor to the receptor plant could occur. Even with direct undisturbed grafts, translocation did not always take place. It hardly could be expected that the stimulus would cross a nonliving membrane if it failed to cross a tissue-to-tissue contact. These results are in harmony with those of MOSHKOV (5), who failed to secure transfer of the floral stimulus from the donor to the receptor in *Perilla* unless the scion had been kept on the stock more than 10 days. Tissue union occurred by 9–12 days, and MOSHKOV concluded that such union was necessary before the transfer of the floral stimulus could occur.

The floral stimulus in *Xanthium* moves both up and down the stem, as evidenced

by the fact that flowering takes place both above and below the point of induction. Since the translocation gradient through the xylem of plants is usually upward, it appears unlikely that any downward movement of the floral stimulus would occur in this tissue. Further, HAMNER and BONNER (4) have shown that placing plants in a saturated atmosphere did not affect the flowering in *Xanthium*, indicating that it was not dependent upon the transpiration stream.

In the present experiments the failure of the stimulus to cross the dead petiole section whose xylem vessels were functional all during the induction period, as evidenced by the turgid condition of the leaf, made it apparent that the stimulus did not move down the petiole in nonliving cells or through functional xylem. This is in harmony with results of other workers. When BORTHWICK and PARKER (1) maintained low temperatures around a section of the petiole of a Biloxi soybean leaf during induction, or around the stem immediately below the apical bud, the plant failed to form floral primordia. This is indicative that living cells were involved in the translocation of the floral stimulus from the induced plant portion to the bud and that the low temperature inhibited the activities of the living cells, thus preventing the transport of the floral stimulus. CAJLACHJAN (3) used low temperatures and narcotic applications on stem portions of *Perilla* and was able to prevent translocation of the floral stimulus from the induced plant portion. He concluded that living cells were involved in the translocation of the floral stimulus.

The experiments in which the bark was removed, leaving intact and functional xylem and pith, show that the floral stimulus does not move either up or down the stem in the xylem or pith to any appreciable extent but moves chiefly in the bark. It would appear likely that the floral stimulus is translocated in the phloem, since this is the tissue primarily concerned with translocation. In some cases, plants with half-sections of the stem removed failed to flower, probably because sufficient stimulus to cause flowering was not translocated before removal of the donor branch.

It generally has been assumed that the effects of girdling are due to the accumulation of carbohydrates above the girdled portion of the stem. Since girdling also appears to block the movement of the floral stimulus, it may be that the effects of girdling on flowering, as reported by ROBERTS and STRUCKMEYER (6) and others, are due to interference with the movement of factors controlling phasic development as well as to interference with the movement of carbohydrates.

Summary

1. The floral stimulus in *Xanthium pennsylvanicum* crossed an inarch contact between an induced donor plant and a receptor plant only when tissue union was established and direct tissue contact was uninterrupted for more than 4 days.

2. The floral stimulus failed to be translocated downward from an induced leaf through dead petiole tissue or through functional xylem cells.

3. The floral stimulus failed to be translocated from an induced donor branch of a two-branched plant to a receptor branch when the bark was removed from the donor branch below the induced leaves or from the receptor branch immediately above the point of branching.

4. The floral stimulus is translocated chiefly in the bark, both up and down the stem.

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NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF CONVOLVULUS ARVENSIS[1]

JOHN C. FRAZIER

(WITH NINE FIGURES)

Introduction

In view of the seriousness of the weed situation and the desirability of a greater knowledge of the characteristics and habits of individual kinds of weeds, in order to combat them most efficiently, a study of field bindweed, *Convolvulus arvensis* L., was undertaken. According to HEGI (3) this plant is a native of Eurasia and is now extensively distributed, especially in the temperate zone of Eurasia and North America. It is especially troublesome in regions of limited rainfall because it competes so successfully with cultivated plants for soil moisture. It is perhaps the most noxious weed of the central United States.

GUNTHER (2) states that *C. arvensis* was described by DIOSCORIDES in his Herbal, which was compiled in the first century. According to SACHS (11), HIERONYMUS BOCK described this plant in his Herbal of the sixteenth century.

MALPIGHI was the first (1681) to report the development of adventitious buds on the roots of herbaceous plants, a process he described in some detail for an unnamed species of *Convolvulus*, which IRMISCH (5) believed was *C. arvensis*. Details regarding germination, general nature, rate of development, and regeneration of *C. arvensis* and *C. sepium* were given by IRMISCH, who lamented that in the older and newer descriptions of that day the subterranean parts either received little consideration or were not studied with exactness. He stated that the misconception that herbaceous roots could not bear adventitious buds would not have prevailed so long had other workers not disregarded MALPIGHI's work.

LUND and ROSTRUP (9) studied the root system of Canada thistle, *Cirsium arvense*. They described a type of development which involves lateral roots that grow horizontally for a greater or lesser distance and then turn downward into the soil, with new lateral roots arising at the bend to continue the plant horizontally.

ROGERS, DURRELL, and DANIELS (10) established the following facts regarding *Convolvulus arvensis:* (*a*) that the horizontal roots extend some 2–4 feet and then grow down into the subsoil to the water table, unless the latter is too deep; (*b*) that at the point of turning down a new horizontal root arises and continues in the same general direction; and (*c*) that buds on the root at the bend develop shoot growth.

KENNEDY and CRAFTS (6) called attention to the influence of external factors on the growth habit, especially the relation of favorable soil conditions to the development of permanent lateral roots. They concluded that the marked ability of this plant to produce buds in great numbers is correlated with the fact that the root has many live cells in close proximity to an extensive supply of stored food.

The observations of BAKKE (1),

[1] Contribution no. 439, Department of Botany, Kansas Agricultural Experiment Station.

HITCHCOCK and CLOTHIER (4), KIESSELBACH *et al.* (7), and KILTZ (8) on the nature of the vertical and horizontal portions of this root system agree with the findings of this study.

Observations

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the nature of which is described in the following profile description supplied by the late Dr. W. H. METZGER, of Kansas State College:

Depth 0–15 inches.—A dark brown, granular silt loam; structure not strongly developed; organic matter content medium; loose and friable.

Depth 15–21 inches.—A dark brown silt loam but slightly lighter in color than preceding layer; structure particles larger than in layer above and tending to group into columns.

Depth 21–29 inches.—A brown silty clay loam, more compact than preceding layers; low in organic matter; probably the least permeable layer of the profile but should permit relatively free development of roots.

Depth 39–78 inches.—A brown to grayish brown silt loam containing a network of lime streaks; fragmental structure weakly developed; slight tendency toward columns; more friable than layer above.

Depth 78–144 inches.—Brown silt loam with less lime than layer above, except in lowest 6 inches; very weakly columnar.

Depth 12–18 feet.—Grayish brown silt loam, high in lime content; very pervious.

Depth 18–21 feet.—Brown and slightly finer textured than layer above, probably just barely within silty clay loam class; pervious and containing less lime than at 12–18 feet.

Depth 21–23 feet.—Very much like that at 18–21 feet, except that grayish brown and occasionally very narrow bands of sand are intermingled with the finer textured material; very pervious.

Depth 23–24 feet.—Mottled gray and brown, the brown occurring in streaks and having a rusty appearance, indicating iron; sand layers, somewhat wider than at 21–23 feet, intermingle with silt loam; very pervious and mellow.

No layer of this profile appears to be of such nature as to impede seriously the development of the roots of deep-rooting plants.

METEOROLOGICAL DATA.—The monthly and annual temperatures and precipitation for the first 10 months of 1937 compared with the 81-year average (1858–1938) of these two factors are given in table 1.

TABLE 1

MONTHLY AND ANNUAL TEMPERATURES (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1937

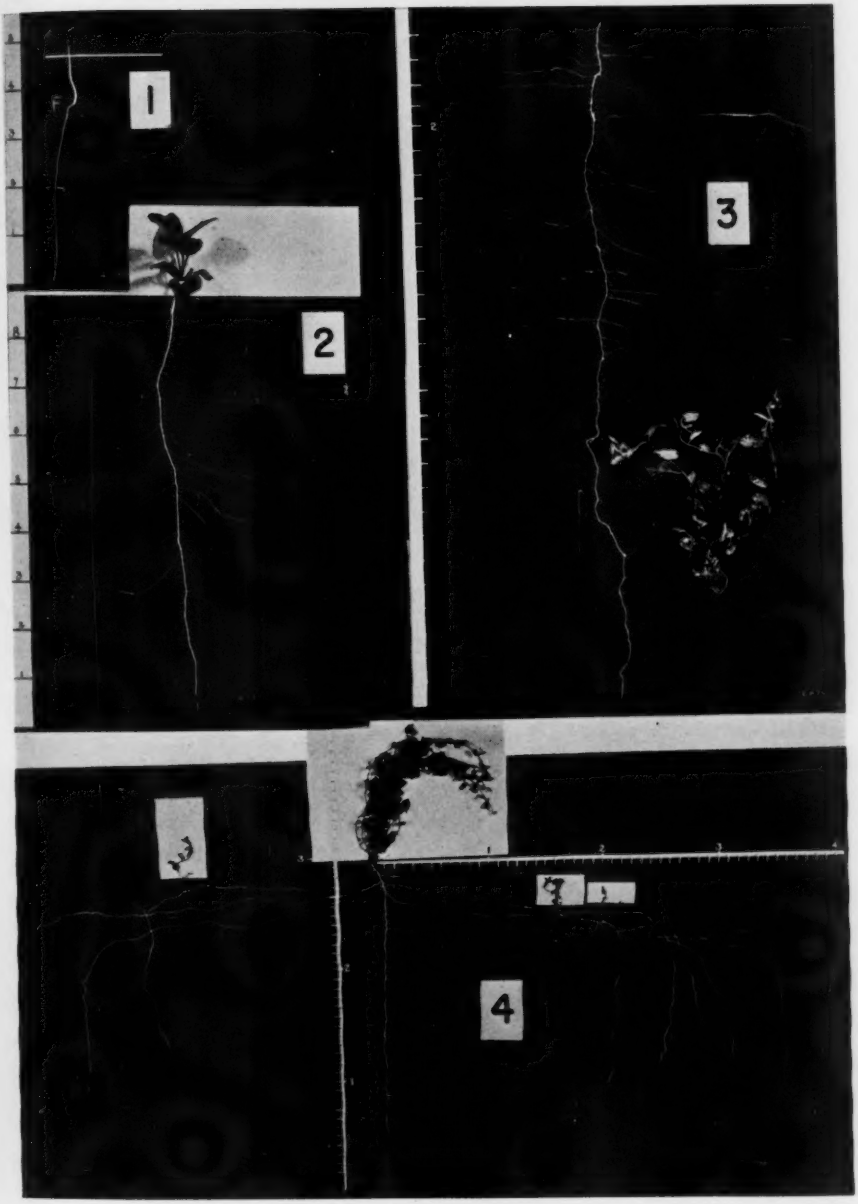
MONTH	TEMPERATURE		PRECIPITATION	
	Mean*	81-year average (1858–1938)†	Total*	81-year average (1858–1938)†
Jan.	21.6	27.3	1.76	0.75
Feb.	30.6	31.5	0.91	1.14
Mar.	39.6	42.5	1.75	1.47
Apr.	54.8	54.6	0.54	2.66
May	67.2	64.7	2.42	4.34
June	76.0	74.5	3.39	4.47
July	82.9	79.8	4.39	4.32
Aug.	84.6	78.2	2.05	3.72
Sept.	72.6	69.9	1.52	3.49
Oct.	56.6	57.1	2.15	2.22
Annual mean or total	54.8	54.48	21.81	30.94

* Meteorological data obtained from U.S. Weather Bureau, Kansas Section.

† Computed by Dr. A. B. CARDWELL, of Kansas State College.

The year 1936 immediately preceding this investigation was one of temperature extremes. The 30-day period ending February 21, 1936, was the coldest on record. That summer was the second hottest on record. There were 60 days when the maximum temperature reached 100° F. or above, during June, July, August, and September. The average number of days per year at this temperature for the last 50 years (1889–1938) is 15. The 1936 rainfall was deficient 6.4 inches as compared with the 81-year average.

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems, commonly called plants in control studies.



FIGS. 1–4.—Stages in development of plants of field bindweed: Fig. 1, seedling 1 week after emergence; *F*, root-stem transition zone. Fig. 2, young plant 4 weeks after emergence (not all vertical root obtained). Fig. 3, 9 weeks after emergence (shoots and roots separately). Fig. 4, 11 weeks after emergence; lateral roots arranged to show development of rhizomes, shoots, and permanent lateral roots of second order; hence, some laterals appear to be above the shoots of others. Figs. 1, 2, scale in inches; figs. 3, 4, scale in feet and inches.

January, 1937, was the fifth coldest January in 50 years. The following summer was hot, with temperatures of 100° F. or higher prevailing for 38 days. January, 1937, had more than 1 inch of precipitation above the 81-year average. March and July were the only other months that exceeded this long-time average. The average monthly precipitation for the first 10 months of 1937 was 2.09 inches as compared with the monthly average of 2.86 inches for these months during this long-time period.

Methods

A small plot of land free of both bindweed and sodium chlorate was used. The soil profile, already given, showed a rather typical Geary silt loam, representative of the wind-deposited soil of the upland of this area. The plot had been cultivated but was undisturbed otherwise.

Plantings were made in late October, 1936. Fifteen seeds were placed 1 inch below the surface at each of twenty-five points, which were so spaced that eight of the plantings were 18 feet distant from any other and the remainder were separated one from another by 8–9 feet.

Emergence of seedlings began April 4, 1937, and on April 9 at least one seedling emerged at twenty of the twenty-five points. All other seedlings were removed, as were those emerging after that date. All plants shown in the accompanying illustrations emerged on April 9. The area was kept free of other vegetation, so the only competition for water and nutrients, if any existed, was among the bindweed plants.

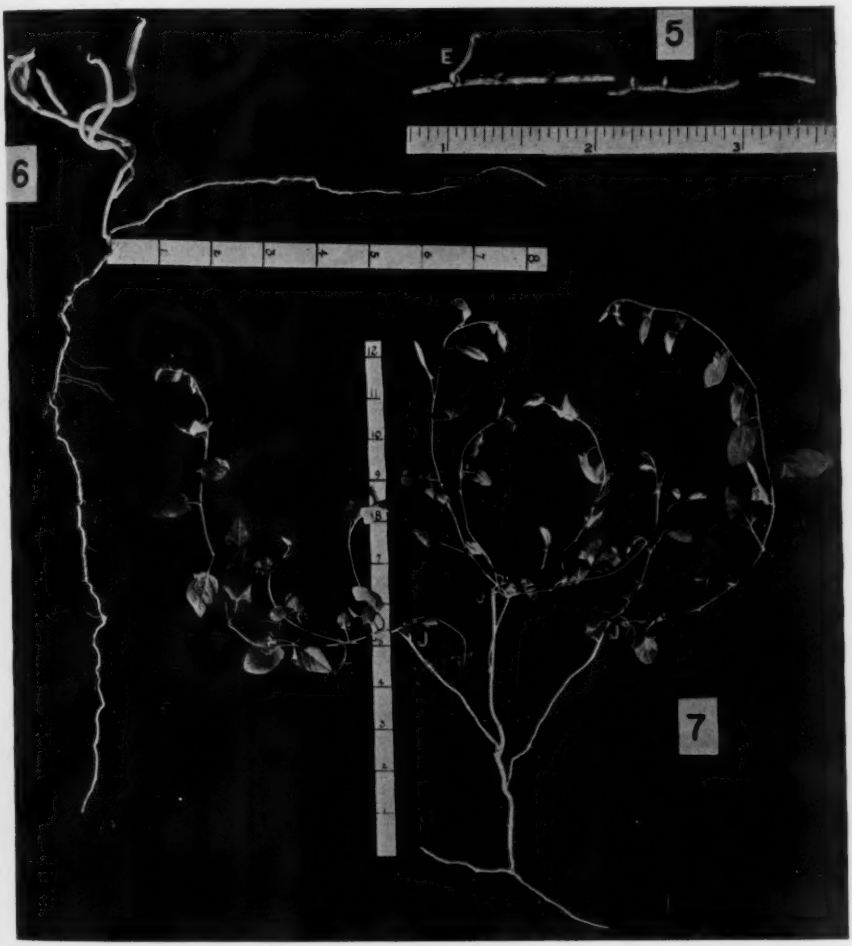
The root systems were excavated by a modification of the trenching method developed by WEAVER (12), which involved the use of a hand-pick and an ice-pick to expose and free the underground parts, after suitable trenches had been made.

Effort was made to keep the root system as one organic entity, hence, when it was broken, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, and the relationship of parts noted as the root system was excavated, so that the plant as illustrated occupied the same relative relationship, one part to another, as it did in the soil (except that figures 2–8 have all lateral roots arranged in one plane). While as many of the finer roots were obtained as possible, it is not contended that many were secured, except for the plant shown in figure 2, in which case a fine jet of water was employed to free the roots from the soil.

Results

Plants were excavated at weekly intervals for the first 11 weeks, and also the thirteenth and the twenty-ninth weeks after emergence. The plant taken the thirteenth week after emergence (fig. 8) showed its gross morphological nature particularly well, especially the root system. A taproot rapidly penetrated directly down from the germinating seed. It was a primary root in order of development and vertical in position, and hence is designated the primary vertical root (fig. 8*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably located, grew extensively and became permanent parts of the root system. They are designated permanent lateral roots (fig. 8*L*). They grew horizontally 15–30 inches and then turned down in a broad sweeping bend and were from that point vertical taproots, designated secondary vertical roots (fig. 8*S*). The horizontal portions of the lateral-vertical roots of any order, which are usually smallest in diameter at their origin, were nearly always in the surface foot, more commonly in the upper 4–6 inches of the soil.

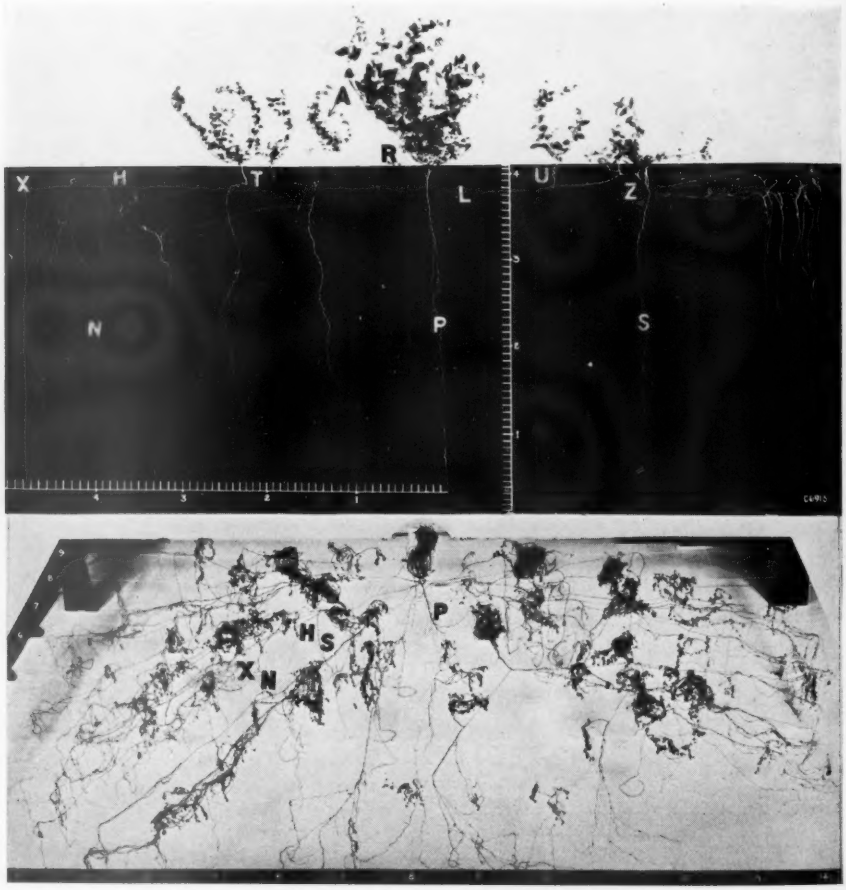
These lateral-vertical roots of the first order gave rise to branch roots over any part of their horizontal or vertical extent. Most of them remained as small feeding roots. However, at or just below the bend where the permanent lateral roots became secondary vertical roots, permanent laterals of the second order formed (fig. 8*H*). They extended horizontally 10–20 inches from their point of origin, the general direction of growth being away from the primary vertical root, and turned down to form secondary vertical roots of the second order (fig. 8*N*). This development was repeated many times during the growing season to give rise to



FIGS. 5–7.—Fig. 5, permanent lateral root showing development of root-borne stem buds, which if borne underground give rise to rhizomes (*E*). Fig. 6, heavy rhizome arising at juncture of permanent lateral and secondary vertical roots, showing scale leaves at nodes and branching. Fig. 7, short section of permanent lateral root bearing branched vertical rhizome, upper parts of which are leafy stems above ground line (*J*). Scales in inches.

lateral-verticals of the third, fourth, and even higher orders. This is suggested at *X* of figure 8 and is shown to advantage in figure 9. It is by such series of permanent lateral roots that horizontal spreading of the plant is accomplished.

If more than one permanent lateral root arose from the place of origin of such roots of the second or higher order (which is on or just below the bend separating the permanent lateral root of any order from its vertical phase), they radiated somewhat from the point of origin in their growth; but in general all maintained growth away from the primary vertical root. This resulted in the plant's



FIGS. 8, 9.—Fig. 8 (above), complete plant 13 weeks after emergence. *P*, primary vertical root; *L*, permanent lateral root of first order; *S*, secondary vertical root of first order; *H*, permanent lateral root of second order; *N*, secondary vertical root of second order; *X*, permanent lateral root of third order; *T* and *Z*, heavy rhizome and shoot development on or near juncture of permanent lateral and secondary vertical roots; *A*, the one flower on this plant; *U*, rhizome arising from lateral root; *R*, shoot from rhizome borne in primary vertical root. Scale in feet and inches. Fig. 9 (below), approximately half a plant 29 weeks after emergence. The growth, supported by strings placed at foot intervals on a 10 × 16 foot frame, is at such an angle that some roots may appear to be above some of the shoots. Scale in feet.

occupying a higher proportion of the ever-increasing area than it would have otherwise. This area was somewhat circular in shape.

All shoot development, except that from the plumule, was derived from root-borne stem buds. These developed on any part of the permanent root system (that is, the two types of vertical roots and the permanent lateral roots). The shoot growth shown directly above the primary vertical root in figure 8 is made up predominantly of stems originating from root-borne buds formed on the primary vertical root in the upper inch or two of soil. This occurred in spite of the fact that the original growth from the plumule was augmented by the development of axes from its cotyledonary and other nodes.

The location of heaviest shoot development from root-borne stem buds was at the bend separating the lateral root of any order from its vertical phase (fig. 8*T*, *Z*). This was the site of heavy shoot development in the older plant shown in figure 9. Less commonly, shoots were found along the horizontal portion of permanent lateral roots (fig. 8*U*).

Regardless of where these root-borne buds formed, if they developed they gave

TABLE 2

RATE AND NATURE OF DEVELOPMENT OF PLANTS OF BINDWEED AT INDICATED INTERVALS AFTER SEEDLING EMERGENCE; MANHATTAN, KANSAS, 1937

Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	4¾	.....	Cotyledons distinctly shaped and incised on adaxial side (fig. 1)
2	6¼	2	First two true leaves
3	9	3	Additional true leaves; cotyledons still present
4	9	3¾	Primary stem axis beginning to elongate (fig. 2)
5	11	4¼	Cotyledonary buds developing shoots
6	12¼	4½	Other axillary buds becoming active
7	13¼	7	Primary stem axis markedly elongated and nutating for support; cotyledons withering
8	18	7½	All secondary stem axes elongating rapidly; cotyledons lost
9	28½	10½	Primary and secondary stem axes 7–8 inches long
10	34	21½	First root-borne stem bud on lateral root 13 inches from primary vertical root
11	36	42	Root-borne stem buds (fig. 4) have developed rhizomes shown in detail in figs. 5 and 6. Three of the 52 rhizomes have leafy shoots (fig. 7)
13	49½	63	Flower in bloom (fig. 8*A*); permanent lateral roots of first (*L*), second (*H*), third (*X*) orders. Typical bend shown at *T* and *Z*, with rhizomes and their shoots and lateral roots of second order (*H*). Sixty-three rhizomes beyond bud stage, many of them on laterals of second order (to right of *Z*). Thirteen rhizomes bore leafy shoots, two on permanent lateral roots of second order. Shoot borne by rhizome (*R*) on primary vertical root
29	46	10 ft., 4	Approximately half a plant (fig. 9). Complete plant had twenty permanent lateral roots of first order, ten of which are shown with almost all their growth and with place relationship of parts essentially as they were in the soil*
120†	23 ft., 1½	At least 16–17 ft.	Secondary vertical roots extended 16–18 feet, with one traced to more than 23 feet‡

* No other plant studied had more than twelve permanent lateral roots of first order.

† 1939.

‡ No primary vertical root was found deeper than 14 feet 8 inches.

rise to rhizomes, unless the buds were borne at or above the ground line, in which case they gave rise to leafy shoots. Figure 5 shows such a series (right to left) by which these buds develop into rhizomes (*E*). Figure 6 shows a well-developed rhizome which has branched, but no branch of which has reached the surface, with the location of its nodes indicated by the scale leaves. In the axils of these leaves are buds which, if they develop, form branch rhizomes (unless borne at or above the ground line). Figure 7 shows a short section of permanent lateral root bearing a branched rhizome, the upper portions of which are leafy stems above the ground line (*J*).

The rate of development of the root system under known soil and climatic conditions was observed (in 1937) by means of eleven plants excavated weekly between April 16 and June 24 and a twelfth and a thirteenth plant excavated July 9 and in late October, respectively. A fourteenth plant was excavated in late July, 1939. Figures 1, 2, 3, 4, 8, and 9 show the general vertical penetration and radial spread of representative plants. Data of the fourteen excavations are given in table 2. These plants emerged on the same date, and the first eleven excavations were separated by the same interval of time; yet differentials in the rate of growth caused unequal degrees of development in the series; for example, the plant taken on the eleventh week appears to have reached a stage of development beyond what would be indicated by the plant taken 10 weeks after emergence.

Discussion

The rate of growth of plants of field bindweed from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential under this favorable noncompetitive situation.

No soil layer was found which would seriously impede vertical penetration of the roots. However, the roots were smaller in diameter in the 34–39-inch portion of the 21–39-inch interval than they were in the 15 inches above or below this portion. In the study made the 120th week after emergence, practically all the vertical roots observed penetrated this 34–39-inch layer, and the only modification noted was the smaller diameter in the layer. Apparently the general nature and rate of growth found in these studies is representative.

The plants obtained the fourth, fifth, sixth, and seventh weeks after emergence were not secured in their entirety, particularly the full vertical penetration of their roots, owing to the methods used in excavation. Consequently, the experiment for the first 7 weeks was repeated the spring of 1942, which was a better growing season because of more favorable moisture relations. The findings were similar, except in regard to maximum vertical penetration and radial spread. The plant 4 weeks after emergence penetrated vertically $11\frac{3}{4}$ inches with a $4\frac{1}{4}$-inch radial spread; the fifth-week plant reached a depth of $14\frac{1}{2}$ inches with a radial spread of 8 inches; the sixth-week plant extended $17\frac{1}{2}$ inches vertically and 10 inches radially; while the measurements for the plant 7 weeks after emergence were $19\frac{1}{2}$ and $12\frac{1}{2}$ inches, respectively.

KENNEDY and CRAFTS (6) state that the lateral roots which develop extensively to become permanent lateral roots are those more favorably located. This investigation offers no better explanation.

Summary

1. Field bindweed plants were grown from seed in a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions, and not subject to competition. The plants were studied at various ages, from the seedling stage through 120 weeks of growth, to determine their nature and rate of development.

2. The root system consisted of the original root (primary vertical root) and one to many permanent lateral roots, the vertical extensions of which are termed secondary vertical roots. Unless injury or too severe competition prevents, there are many additional permanent lateral and secondary vertical roots of the second and higher orders.

3. The plant spreads horizontally by means of series of permanent lateral roots. The primary permanent lateral roots arise on the primary vertical root. Succeeding orders of permanent lateral roots arise at the bend where a permanent lateral root of the preceding order turns down to become a vertical root.

4. The plants spread radially, by means of a series of lateral roots, 10⅓ feet in one growing season and 17 feet by the middle of the third growing season, at which time many vertical roots had extended 14–16 feet deep, and one was traced to a depth of 23 feet.

5. The source of shoot development other than that arising from the plumule was determined as root-borne buds which form leafy shoots directly (if at or above the ground line) or rhizomes (if below ground), which in turn give rise to leafy shoots. These arose in greatest abundance at the bend separating the lateral root of any order from its vertical phase. The shoot development of old plants is wholly from root-borne buds.

The writer is indebted to members of the departments of botany of the University of Chicago and of the Kansas Agricultural Experiment Station for helpful suggestions during the course of this study.

DEPARTMENT OF BOTANY
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MANHATTAN, KANSAS

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DEVELOPMENTAL ANATOMY OF THE FRUIT OF THE DEGLET NOOR DATE[1]

E. M. LONG[2]

(WITH NINETEEN FIGURES)

Introduction

Quality, as well as size, of mature fruit of the Deglet Noor date is influenced by cultural operations, during the period of fruit development. NIXON and CRAWFORD (6, 7) showed that heavy, as compared with medium, thinning of the fruit clusters or bunches increased susceptibility of fruit to checking during midsummer but decreased fruit shrivel during ripening. (Checks are minute cracks which form in the cuticle and extend through the epidermis and first two to four tiers of the cells of the hypodermal layer. Shrivel appears during ripening, the mesocarp apparently decreasing in volume excessively, so that a wrinkled outer surface develops, as illustrated in figure 8.) ALDRICH *et al.* (1) found that soil-moisture deficiency in June resulted in a greater reduction in rate of fresh- than of dry-weight increase, a reduction in susceptibility of fruit to checking in July and early August, and a tendency for increased shrivel of fruit during ripening. These and other results are more readily interpreted on the basis of chemical and anatomical changes during development.

HAAS and BLISS (4) and RYGG (9) investigated chemical changes during fruit development, but information on the developmental anatomy of the fruit has been incomplete. WINTON and WINTON (13) briefly discussed the structure of the mature fruit, and LLOYD (5) supplied some data on anatomical changes during development, although he was primarily interested in nutrition of the various parts of the fruit. HAAS and BLISS (4) demonstrated that most of the growth in length occurred in the basal region of the fruit, and TURRELL *et al.* (12) studied maturation of the tissue layers of the exocarp in relation to fungus spoilage.

Material and methods

The carpellate inflorescence of the date is a branched spadix composed of 25 to 100 or more spikes, 6–36 inches in length, and may have 8,000–10,000 flowers (8). Material was obtained from inflorescences pollinated March 18, 1941, at weekly intervals from four vigorous young palms at the U.S. Date Garden at Indio, California. At the time of pollination many spikes were entirely removed and several inches taken off the ends of those remaining.

Available soil moisture was maintained by irrigation during the period of active growth. A representative strand was photographed each week for a record. The material for anatomical analysis was fixed in Navashin's solution, dehydrated in an ethyl-tertiary butyl alcohol series, and infiltrated and imbedded with a paraffin-beeswax-rubber mixture. Immediately after placing in the fixative, air was evacuated from the tissues with a vacuum pump. The material was sec-

[1] This study was financed by the United States Date Garden, Indio, California, and was prosecuted under the joint direction of Dr. W. W. ALDRICH, now of the U.S. Horticultural Field Laboratory, Riverside, California, and Dr. H. E. HAYWARD of the U.S. Regional Salinity Laboratory, Riverside, California.

[2] Agent.

tioned at 6–10 μ and stained by ESAU's (3) modification of the iron-alum haematoxylin schedule.[3]

meristem two or possibly more years previous to spathe emergence. The apical bud is found several feet below the

FIGS. 1–8.—Carpellate flowers and fruits from soon after pollination to fruit ripening: 1, March 27 (early in period I); 2, April 17 (late I); 3, May 8 (early II); 4, May 29 (early III); 5, June 12 (late III); 6, July 17 (early V); 7, August 28 (early VI); 8, October 12 (early VII). Period numbers as shown in fig. 21. All marks on fruits shown in figs. 4 and 5 and mark near equator of fruit in fig. 6 made with ink May 28; mark near base of fruit in fig. 6 made June 19. All marks on fruits in fig. 7 made ½ inch apart on June 26.

Results

FIELD OBSERVATIONS

The inflorescences are borne in large, tough, leathery spathes which emerge from the axils of leaves expanded the previous year, the primordia of which must have originated from the apical level of the expanding leaves, closely and completely surrounded by their sheathing bases, which render the bud almost inaccessible. The carpellate flower is trimerous and ivory white at emergence. The three carpels are not adnate but closely appressed and almost wholly inclosed by a coriaceous corolla composed of three closely imbricated petals (fig. 1).

[3] Most of the slides were prepared by Mr. ARTHUR SPURR.

The calyx is also coriaceous and composed of three thick sepals, of which only the tips are diverged. After emergence, the color of the flower changes through cream and yellow to green.

In 1941, growth was very slow during the first 4 weeks after pollination. By the end of that period one carpel usually became ascendant over the other two, which aborted (fig. 2). During the fifth week after pollination the dominant carpel began to enlarge (fig. 3), and as it emerged from the surrounding perianth, the calyx was split at one or more points.

To demonstrate the relative growth rates in different regions of the carpel, fruits were marked on May 28, the end of the tenth week after pollination, with India ink (4). A heavy black line was made at the base of the carpel, overlapping the distal margin of the corolla. Subsequent growth at the base separated the portion of the mark on the carpel from that on the corolla. The fruits in figures 4 and 5 were photographed 1 and 14 days, respectively, after marking and demonstrate the rapid enlargement during the eleventh and twelfth weeks after pollination. Although growth in length occurred both above and below the marks, by far the greater proportionate longitudinal extension was in the basal region.

Several fruits that had been marked on May 28 were marked again (ink overlapping both carpel and corolla) on June 19. Between June 19 and July 17 (fourteenth through seventeenth week) both median and basal portions of the fruit grew in length, the basal more than the median portion (cf. figs. 5, 6). The distance between the mark of June 19 and the tops of the corolla increased at a diminishing rate until about August 28.

By August 28 the fruits (fig. 7) had attained maximum size and the pink color characteristic of the "khalal" or preripe stage of the Deglet Noor variety. The marks on the fruits shown in figure 7 had been made $\frac{1}{8}$ inch apart on June 26. On August 28 there was no measurable increase in circumference in the apical portion of the fruit after June 26. Growth in circumference was about equal in both median and basal portions. Growth in length during the June 26–August 28 period occurred only in the basal two-thirds to three-fourths, with progressively less growth at increasing distances from the perianth.

During September the fruit decreased in size as a result of dehydration incident to ripening (1, 4, 9). As the pink color changed to brown, softening began at the tip. Fruits on the same strand began to soften at different times during a 2-month period beginning the last week in September. The fruit in the lower part of figure 8 was ripening. It shows some shrivel, which is commercially undesirable but typical for the district in which this fruit was grown.

Detailed observations

Six rudimentary stamens regularly occurred in carpellate flowers, although no anthers were observed. At time of pollination, comparatively little cellular differentiation was evident in the carpel (figs. 9, 10). Differentiation, beginning at the apical end, progressed slowly for 1 month thereafter, then more rapidly. By the seventh week all tissue layers were well differentiated at the equatorial region (fig. 11), and cell division in the mesocarp at that level had been completed, further growth of that tissue being accounted for wholly by cell enlargement.

To encompass the increase in volume of mesocarp from the seventh week to attainment of maximum size, the exo-

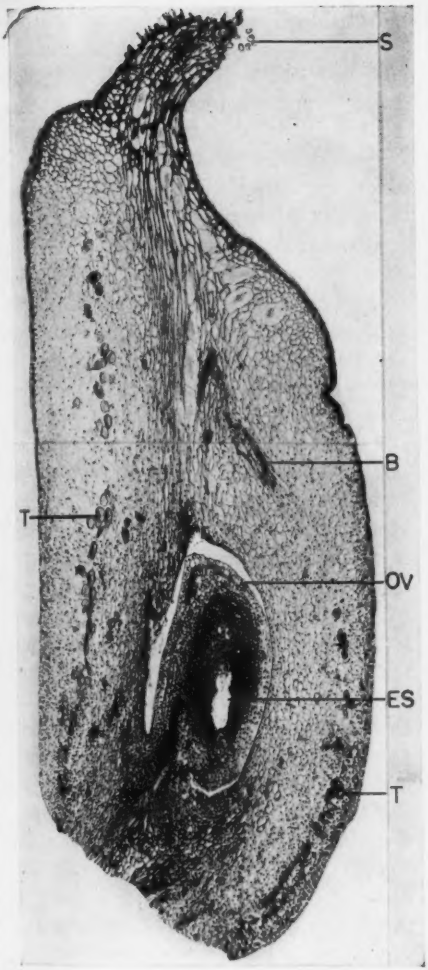


FIG. 9.—Nearly median longisection of carpel at pollination, March 18 (period I): *s*, stigma; *b*, abaxial bundle; *ov*, ovule; *es*, embryo sac; *t*, tannin cells.

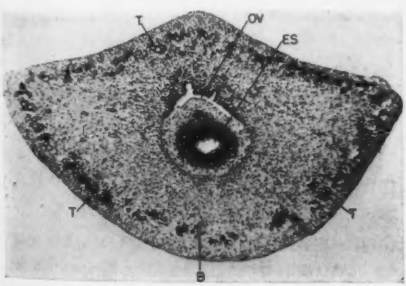


FIG. 10.—Transection of carpel at level of embryo sac.

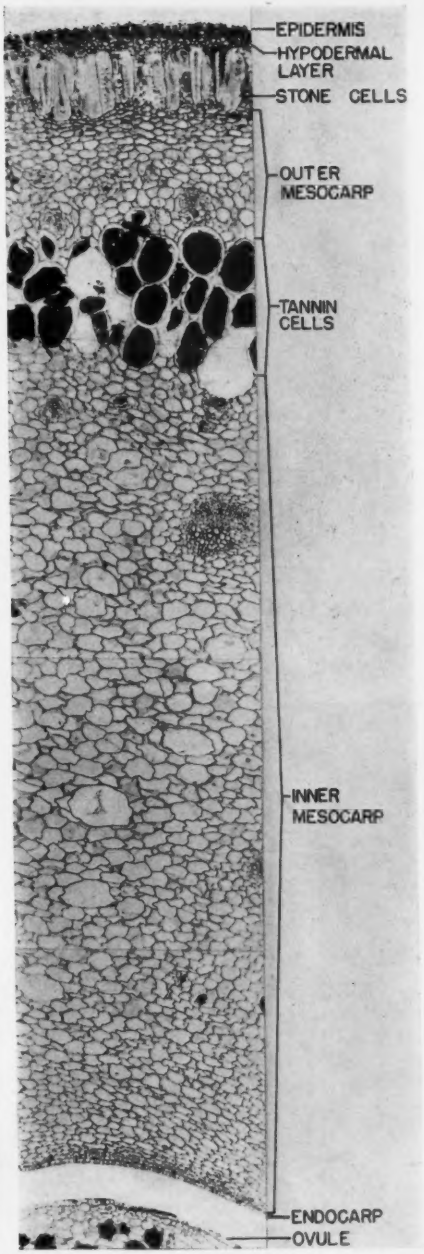
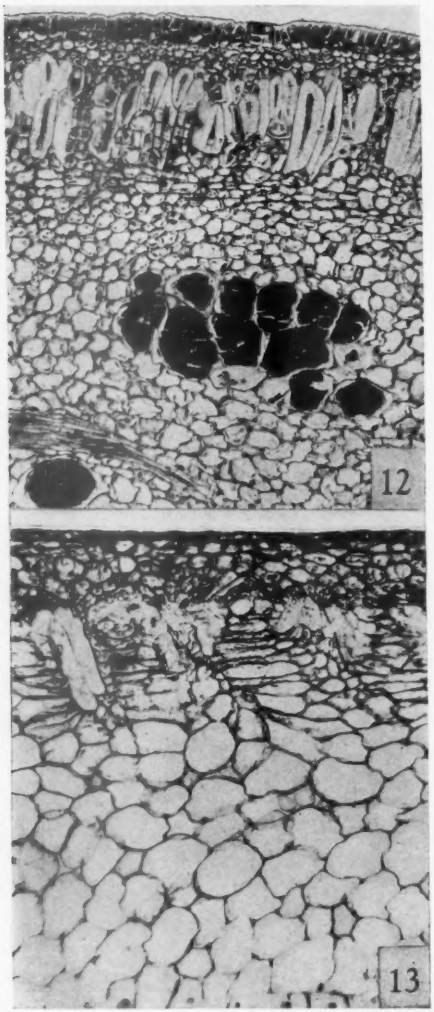


FIG. 11.—Sector of transection through pericarp from equatorial region of fruit collected May 8 (7 weeks after pollination) shortly after completion of cell division in inner mesocarp at this level.

carp must increase in circumference by at least 200 per cent. Longitudinal growth of the exocarp in this zone could not be accurately measured but must have been considerably greater than growth in circumference, for cells of the mesocarp grew more in the longitudinal than in either transverse dimension. Also, the minute cracks or checks which may occur in these fruits during rainy or humid weather from mid-June to mid-August are always transverse, seeming to indicate that in this variety tension on the cuticle where the cracks originate (2) is greater in the longitudinal direction.

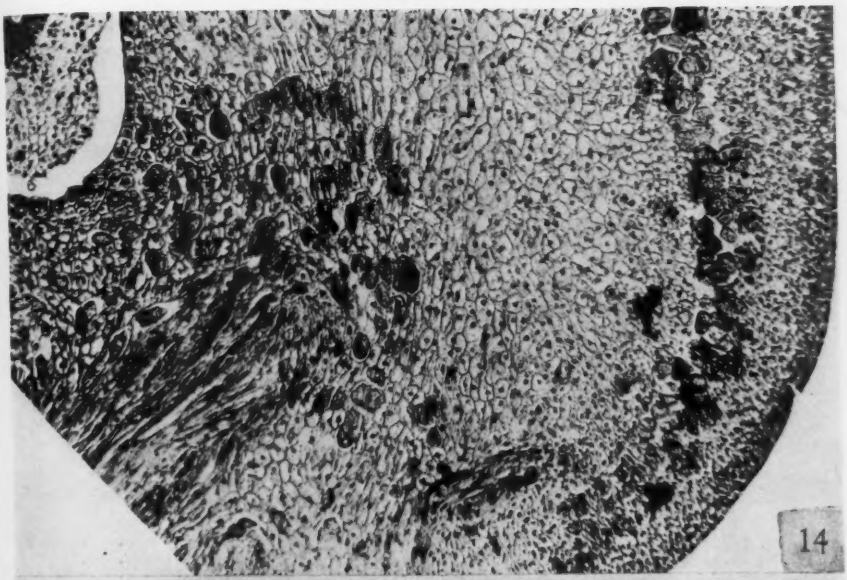


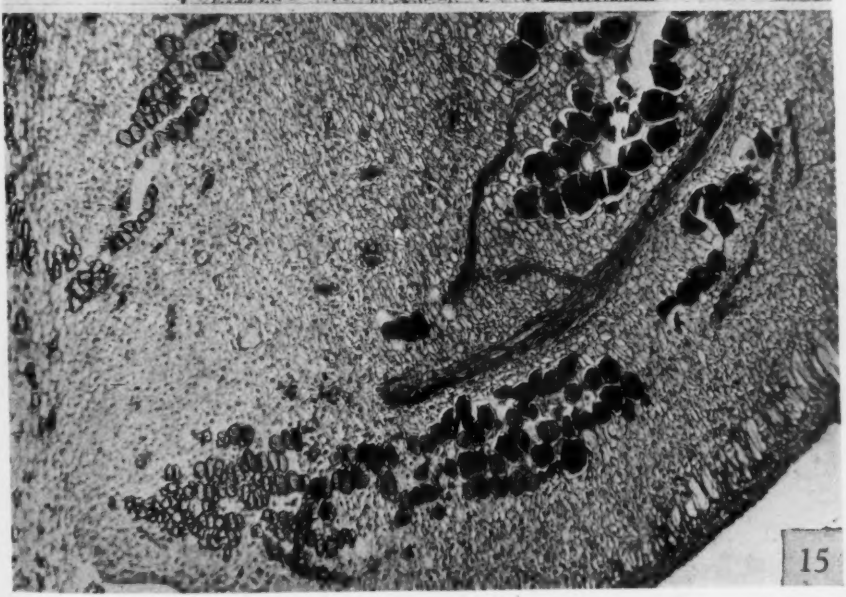
FIGS. 12, 13.—Fig. 12, exocarp and outer mesocarp in radial longisection from equatorial zone of fruit collected May 15. Fig. 13, exocarp and portion of outer mesocarp in radial longisection from apical third of fruit collected September 4, at approximately maximum size, showing extension in stone cell layer accomplished mainly by "stretching" of intervening parenchymatous cells.

Comparison of figures 12 and 13 shows how the exocarp grew in regions of the fruit where tissues were well differentiated. In the epidermal and hypodermal layers both cell division and cell enlargement took place. After stone cells began to mature in a given region, no new stone cells were formed, but the intervening parenchymatous cells elongated greatly, isolating clusters of stone cells. Orientation of many of the stone cells also appeared to change during extension in the exocarp.

Through the first month after pollination little mitotic activity was apparent (fig. 14), and the relatively few cell divisions that did occur were distributed throughout the carpel. This contrasts sharply with the aspect of the basal region 2 weeks later (fig. 15), shortly after organization at the base of the carpel of an actively meristematic zone. Continued activity in this meristem gave rise to cells which constituted more than three-fourths of the mature pericarp.

In some fruits the basipetal meristem ceased activity by the middle of June, while in others it continued active for as much as another month. This was the first indication of a deviation in development of a fruit destined to mature early as contrasted with a later-ripening one. No reason for this difference was appar-

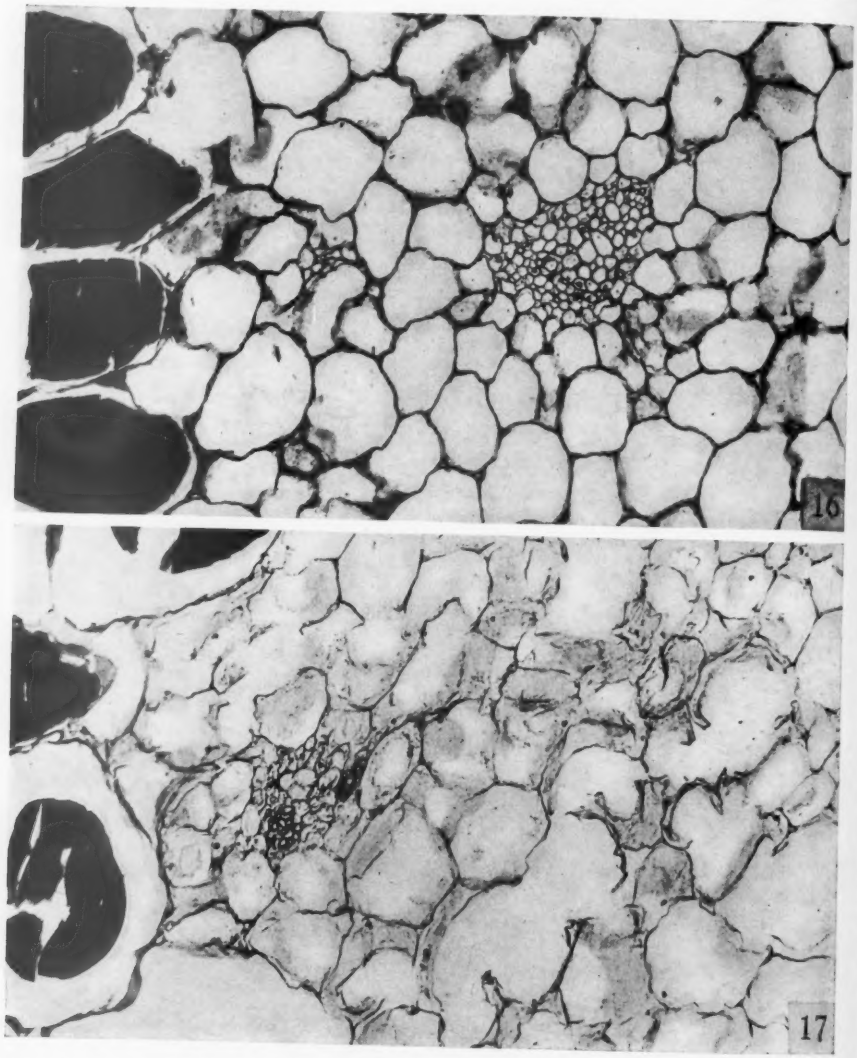




FIGS. 14, 15.—Fig. 14, radial longisection through basal region of fruit collected April 17, showing no evidence of recent, very active cell division, although scattered cell divisions occur throughout carpel at this stage (late period I). Fig. 15, radial longisection through basal region of fruit collected May 1, soon after beginning of activity of basipetal meristem, showing evidence of rapid successive divisions of cells of basal zone.

ent. About the middle of July, cells of the basipetal meristem of even the latest maturing fruits ceased to divide, enlarged, and finally matured.

Besides the color change already mentioned, the most apparent outward evidence of ripening is softening, accompanied by dehydration, so that the fruit becomes somewhat flaccid. The change in texture is due in part to the fact that



FIGS. 16, 17.—Partial dissolution of cell walls which contributes to softening of fruit during ripening. Fig. 16, mesocarp in transection from equatorial zone of fruit collected August 21, nearly full size but immature; cell walls relatively thick and strong (late period V). Fig. 17, comparable tissues of ripe fruit collected October 10; walls thin and often broken (period VII).

the walls of the mesocarp cells become thinner and weaker, apparently being hydrolyzed, until some of them undergo almost complete disintegration (figs. 16, 17). Cell membranes apparently lose the property of differential permeability before microscopic evidence of wall dissolution is apparent (2). Softening begins at the apex of the fruit, progressing toward the base, and also from the periphery toward the endocarp.

CELL COUNTS AND MEASUREMENTS.—To determine the time at which periclinal divisions ceased in the terminal segment of the fruit, cell size was plotted against breadth of mesocarp (fig. 18*B*). This method was suggested by SINNOTT (10), who states the theoretical relationship between cell size and organ size (expressed graphically in fig. 18*A*) thus: "the cessation of cell division is associated with the attainment of a definite cell size. . . . . Increase in cell size is not a continuous process, but is interrupted at each cell division, for cell volume is then reduced by half and cell diameter by slightly more than 20 per cent. . . . . Each daughter cell now increases . . . . to a somewhat greater diameter than that of its mother cell and then divides again. Thus the changes in size in a given cell lineage are not to be represented by a continuous line but by a serrate one. . . . . After the last division, cell enlargement proceeds continuously, uninterrupted by further breaks, at approximately the same rate as fruit enlargement."

In obtaining the data from which curve *B* of figure 18 was plotted, the distance from endocarp to the tannin cells (fig. 11) opposite the chalazal end of the ovule was measured with an eyepiece micrometer. Then the number of cells intersected by the edge of the micrometer scale in the distance measured was counted. Thus many cells were measured at tapered ends, while SINNOTT (10) arbitrarily selected for measurement cells judged to be "a common maximum size . . . . characteristic of the whole area." Owing to the considerable variation in size of cells selected at random, it was necessary to measure many in order to obtain reproducible values for average cell diameters. To compensate for distortion due to compression of the material during sectioning, all counts and measurements were made once in the direction parallel to the knife edge at

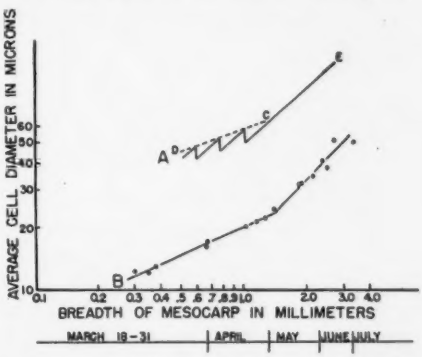


FIG. 18.—Relation of cell diameter to breadth of mesocarp plotted logarithmically. *A*, theoretical curve, after SINNOTT (10). *B*, actual curve for apical segment.

sectioning and again in the direction at right angles to the knife. The values represented by one point in figure 18 are averages of two such counts and measurements for each of three to six fruits, and they include at least 150 cells.

The slope of line *CE* in figure 18*A* is that produced when all the growth of an organ is the result of enlargement of a constant number of constituent cells. In curve *B*, previous to early May the slope is considerably less than that of *CE* (much closer to that of *DC*), indicating that increase in breadth of mesocarp at that time resulted from increase in both number and size of cells. The break in curve *B* in early May, and the subse-

quent slope paralleling *CE*, indicate that after that time all growth in breadth of the mesocarp in the vicinity of the distal end of the seed was due entirely to cell enlargement.

The basipetal growth of the fruit results in so few permanent longitudinally distributed reference points as to make it impossible to locate precisely analogous tissue for study at progressive intervals throughout the season. Since in fruits collected at different times, one could not identify for measurement tissues laid down during any specific period, SINNOTT'S method could not be used to determine the date at which transverse division ceased in any given segment. However, the assumption that "cessation of divisions is associated with the attainment of definite cell size" (10), coupled with adequate cytological observations, permitted rather accurate determination of the role of such divisions in longitudinal growth. To determine growth of cells at any given level, the eyepiece-micrometer scale was superimposed on the middle of the inner layer of mesocarp of a fruit in radial longisection and counts made of the number of cells intersected by the edge of the scale in a measured distance. For each determination a minimum of fifty cells from three to six fruits from one sample were measured. Largely upon the basis of data so obtained (fig. 19), growth and development of the date fruit has been divided into seven periods. TUKEY and YOUNG (11) by a somewhat similar method have divided the development of the fruit of the sour cherry into three stages.

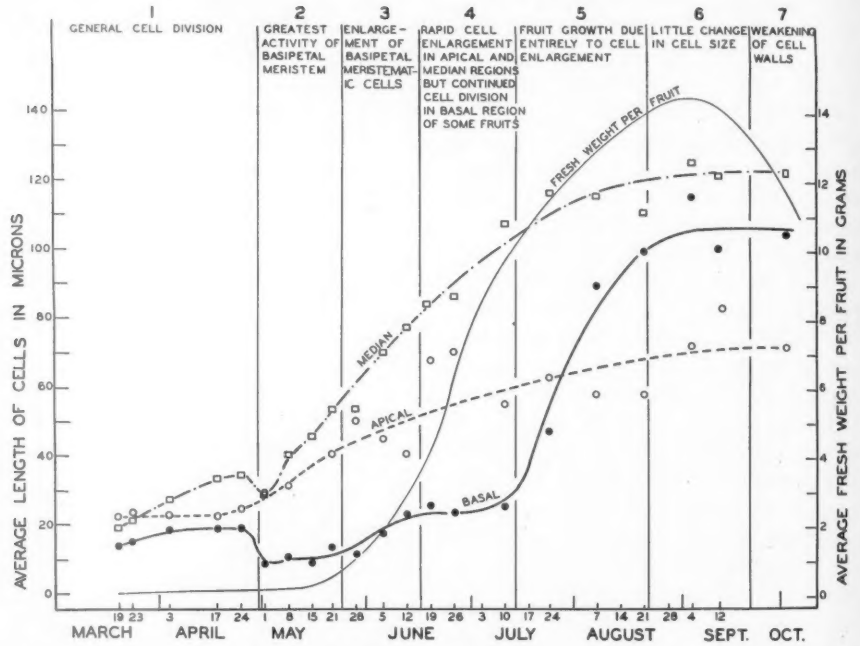


FIG. 19.—Average lengths of cells of basal, median, and apical regions of mesocarp, and fresh weight of average fruits; determinations from pollination to maturation. Data for fresh weight curve provided by W. W. ALDRICH and C. L. CRAWFORD of the U.S. Date Garden.

The curves representing cell length in the basal, median, and apical regions of the mesocarp during period I were more or less similar to *DC* in curve *A* of figure 18, indicating that some transverse cell division was occurring throughout that layer of the pericarp. The second period was initiated by rapid successive transverse divisions of the cells in the basal end of the fruit, resulting in much shorter cells in that region, which then constituted a basipetal meristematic zone (fig. 15). Since the area of active cell division extended at first almost to the equator of the young fruit, the lines representing lengths of both median and basal cells dipped, while apical cell length increased uninterruptedly. Cells in the median zone were rapidly separated from the basipetal meristem by growth of intervening cells; and, after a short phase of mitotic activity early in the second period, they ceased division. During the latter part of period II the endosperm, which had previously been free-nuclear, became cellular.

During period III the cells of the basipetal meristem increased in length, while continuing active division. The end of the period is marked by cessation of division in some fruits, which is associated with and identified by attainment of a cell length of about 30 μ in the basal zone. Thus period IV is initiated by the disappearance of the basipetal meristematic zone in the fruits destined to mature earliest and ends with the cessation of cell division in the basal zones of the latest maturing fruits. Period IV is characterized by the most rapid fresh-weight increase as well as by the beginning maturation of previously meristematic cells. During period V there is no cell division and all increase in the volume of the mesocarp is due to cell enlargement, chiefly of the cells of the basal region.

The stages of fruit development corresponding to periods IV and V have been found by HAAS and BLISS (**4**) and by ALDRICH *et al.* (**2**) to be those during which the fruits are susceptible to checking. Period V corresponds to RYGG's (**9**) stage 2, which he found to be characterized biochemically by a greatly reduced rate of accumulation of reducing sugars and a high degree of hydration.

Period VI, which corresponds in a general way to the "khalal" or preripe stage, is one of little apparent anatomical change. RYGG found that rapid sucrose accumulation and slow dehydration occur at this stage. The fruit is no longer susceptible to checking (**2, 4**), but —if it comes into contact with free water —is subject to tearing, that is, the fruit bursts open as a result of excessive turgor, separation occurs at the tannin cell layer, and segments of the exocarp and outer mesocarp break away from the remainder of the fruit, curling outward at the edges of the rupture.

Period VII is characterized by weakening of cell walls, apparently as a result of hydrolysis (fig. 17), a color change from pink to brown, further increases in sucrose (**9**), and rapid dehydration. Since dehydration decreases the size of the fruit during maturation, at least some of the cells must also have decreased in size. This is not apparent from figure 19, because the material was dehydrated in the histological procedure. Since histological dehydration shrank tissues and constituent cells before they were measured microscopically, actual maximum size of cells was never measured. The decrease in fresh weight, due to water loss during ripening and previous to collection, did not significantly influence values obtained in measuring cell lengths, because the removal of water and consequent volume loss would have occurred during the histological procedure if not previous to it.

Summary

1. The development of the fruit of the Deglet Noor date from pollination to maturation in 1941 was divided into seven periods. The calendar dates for each period refer to the fruits examined in 1941 and may be expected to vary somewhat with the district where grown and in different growing seasons.

2. Period I, from March 18 (pollination) to April 30, is an interval of little enlargement, during which some cell division occurs throughout the mesocarp. Period II, May 1 to 23, is initiated by organization of a meristematic zone at the base of the fruits. Cell division ceased in the apical half of the mesocarp by May 8. At the close of this period the endosperm, previously free-nuclear, became cellular. Period III, May 24 to June 15, is initiated by a sharp increase in rate of enlargement of the entire fruit and all cells of the mesocarp, including those of the basipetal meristem, which, however, continue to divide. At the close of this period the fruits become susceptible to checking (minute cracks involving the cuticle, epidermis, and outer cells of the hypodermal layer). Period IV, June 16 to July 13, begins with inactivation of the basipetal meristem of fruits destined to mature earliest and ends when a meristematic zone is no longer present in any of the fruits. This period is also characterized by the highest rate of fresh-weight increase of the season. Period V, July 14 to August 22, starts with a marked increase in rate of elongation of the previously meristematic cells at the base of the fruits and ends as those cells approach maximum length. At the end of this period the fruit is no longer susceptible to checking, and the color changes from green to pink. Period VI, August 23 to September 21, is distinguished by little apparent anatomical change. Marked increase in percentage of dry matter occurs. Period VII, September 22 to November 3, is characterized by softening as a result of continued dehydration of the fruit and partial dissolution of cell walls. The color changes from pink to brown.

U.S. Date Garden
Indio, California

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DEVELOPMENT OF SORUS AND PROTHALLIUM OF ACROPHORUS STIPELLATUS

BETTY F. THOMSON

(WITH TWENTY-NINE FIGURES)

Introduction

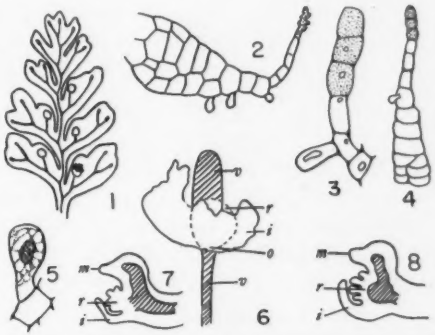
The fern now known as *Acrophorus stipellatus* (Wall.) Moore has been placed in various genera of the Polypodiaceae, including *Davallia*, *Aspidium*, and *Cystopteris*. BOWER (2) discusses it among the Dryopteroids but considers it to be a *genus incertae sedis*. This uncertainty as to its relationships is due chiefly to lack of exact information about the development of the sorus.

The material used in this study was collected in July, 1937, by Professor ALMA G. STOKEY in Java. The plants were growing in rich damp soil at an altitude of 2400 meters. Some specimens were dried and pressed, others were killed and fixed in a formalin-acetic acid-alcohol solution. At the same time prothallial cultures on sterile peat were started from spores. Material for detailed study was later imbedded in paraffin, sectioned at 12 μ, and stained with safranin and fast green or with iron-alum haematoxylin. Living prothallia were also examined whole.

Observations

ADULT PLANT

The plants have an erect stem and a basket-like habit. The leaves are about 1 meter long, quadripinnate, with open, dichotomous venation (fig. 1). Each veinlet ends as an enlarged mass of tracheids which forms an elliptical hump on the under side of the pinnule. The shoot is luxuriantly clothed with hairs and scales of varying degrees of complexity, many of which are glandular. Simple glandular papillate hairs may be found on the leaf surfaces and rachises, and the more complex hairs and scales may have glandular tips or appendages (figs. 2–5).



FIGS. 1–8.—Fig. 1, portion of pinnule showing form, venation, and location of sori. Figs. 2–5, dermal appendages; glandular cells shaded; figs. 3 and 5 more highly magnified. Fig. 6, sorus with sporangia removed showing vein extending beyond receptacle: *v*, vein; *r*, receptacle; *i*, indusium; *o*, point of origin of indusium. Figs. 7, 8, adjacent sections of young sorus, cut parallel to vein, showing receptacle supplied by short branch from vein; vascular tissue shaded; hairs and scales omitted: *m*, recurved leaf margin; *r*, receptacle; *i*, indusium.

Fertile and sterile leaves are alike in their general aspect. Mature sori, one to eight on a pinnule, are situated on the veinlets just short of their swollen ends. The vein may obviously extend past the sorus, or the sorus may appear to cover the entire vein ending. When the sporangia were removed, however, in every specimen examined the vein was found to continue past the receptacle, even when the sporangia extended out in a pompon-

like cluster in such a manner that they concealed the vein ending completely (fig. 6). A branch from the vein passes into the convex, zygomorphic receptacle, where it ends as a slightly enlarged mass of tracheids similar in structure to the enlarged ends of the veinlets in the vegetative parts of the leaf (figs. 7, 8). The basally attached indusium completely covers the young sorus; but as the sporangia enlarge and their stalks elongate, they push the protective scale up and back until it forms an incomplete covering for the sorus.

Development of sorus

The first recognizable indication of a sorus appears when the pinnule is still very small. In cross-section the young leaflet is elliptical, about twice as broad as thick, when the first indications of the vascular strand can be distinguished. At this stage the cells immediately behind the marginal cell on the abaxial side show anticlinal divisions (fig. 10). After further divisions of this nature and growth of the resulting cells, and with the establishment and enlargement of the indusial initial, the young fertile leaflet suggests in section an isosceles triangle, with the apex formed by the marginal initial of the sterile side and the basal corners formed by the large indusial initial and the marginal initial of the fertile side (figs. 11, 12). Because of this triangular shape, the receptacle appears at first glance to be marginal, since the receptacle cells formed by anticlinal divisions push the true margin to an asymmetrical position. But the receptacle is as nearly superficial as possible on a structure so little expanded that it has no flat surface. Figure 9 shows a sterile leaflet at an early stage of development for comparison.

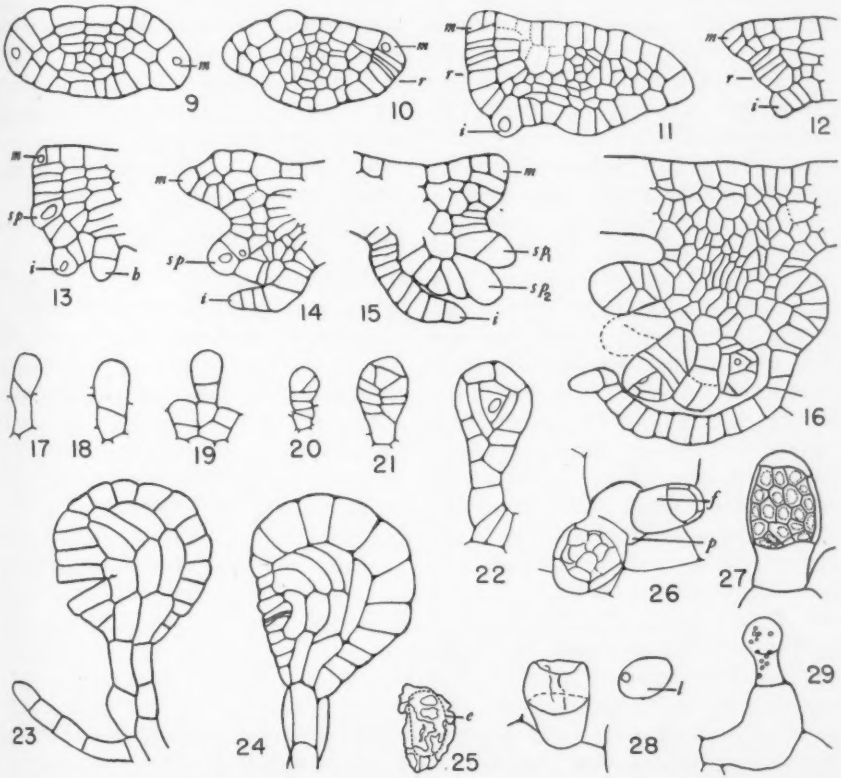
The marginal initial can also be distinguished in later stages (figs. 13–15). No instance was found in which the receptacle could be regarded as truly marginal in origin. In the youngest sorus observed (fig. 10) the latest cell derived from the marginal initial had undergone the anticlinal divisions which lead to development of the receptacle, but in no case was the marginal initial itself involved in the construction of the sorus.

The indusium, developing by means of intercalary divisions, arises superficially just behind the cells which multiply to form the receptacle. It is several cells long as seen in section by the time the first sporangial initial can be distinguished (figs. 13, 14). As the receptacle grows larger and projects farther below the leaf surface, the indusium, with the central part of its base adhering to the receptacle, is carried away from the laminar surface, obscuring the superficial nature of its origin.

The first sporangium arises from the center of the receptacle, which is somewhat convex and tilted toward the leaf margin at this time (figs. 13, 14). The second seems to arise consistently between the first and the indusium (figs. 15, 16). The first four or five sporangia arise in basipetal sequence. The receptacle meanwhile enlarges by means of intercalary divisions and some elongation of the cells at its base and becomes more sharply convex. The sporangia which arise from the later-formed part of the receptacle develop in mixed sequence. They follow one another in such rapid succession that those of a single sorus are all at approximately the same stage of development. The sporangial initial is more or less square at the base as seen in longitudinal section. The first segmenting wall may be transverse but is more often oblique, abutting on a lateral wall of the initial cell, generally—but not al-

ways—below the level of the surface of the receptacle (figs. 17–22). More sporangia are formed on the side of the receptacle toward the indusium than on that toward the leaf margin. Any cell on the surface of the receptacle may become a sporangial initial; in fact, almost every cell over the entire receptacle gives rise to a sporangium, with the result that the sporangia are densely crowded in the mature sorus. Very few paraphyses were found arising from the receptacle; hairs attached to the sporangial stalks are more numerous (fig. 23).

SPORANGIUM AND SPORES.—The mature sporangium is borne on a stalk consisting of three cell rows. The stalk may bear a simple curved hair about four cells long. The capsule is very much like that of *Dryopteris filix-mas* as shown in KNY's *Wandtafeln*. The annulus is vertical and incomplete, being interrupted at



FIGS. 9–29.—Fig. 9, young sterile leaflet, cross section. Figs. 10–16, sections of young fertile leaflets. Fig. 10, earliest stage observed; cells adjacent to marginal initial show anticlinal divisions. Figs. 11–16, later stages: *h*, hair; sp_1, sp_2, first and second sporangial initials. Figs. 17–22, developing sporangia showing variation in initial segmentation. Figs. 23, 24, young sporangia showing unequal faces and hair on sporangial stalk. Fig. 25, spore showing wrinkled perispore: *e*, outline of exine. Fig. 26, two antheridia borne on a pedicel cell (*p*); note cylindrical form of "funnel cell" (*f*). Fig. 27, young antheridium without pedicel cell. Fig. 28, ruptured antheridium with extruded undivided lid cell (*l*). Fig. 29, papillate prothallial hair with chloroplasts, borne on enlarged marginal cell.

the insertion of the stalk (figs. 23, 24). It consists of twelve to fourteen indurated cells and seven to nine thin-walled cells in the stomium region. The stomial cells are elongated transversely and twisted to one side because of the inequality in the numbers of cells on the two faces of the capsule (figs. 23, 24).

The bilateral spores appear black to the eye and golden brown under magnification. The exine is smooth, but the spore is incased in a transparent, loosely-applied membrane or perispore which is folded into an irregular network of ridges, giving the spore a characteristic surface modeling (fig. 25).

During early development of the sorus, the extension of the lamina is delayed, but this lag is subsequently made up, and the blade finally extends far beyond the receptacle. The leaf margin is strongly recurved over the young sorus, which is well developed while the leaf is still tightly rolled. As the leaf unrolls, the blade becomes flattened.

PROTHALLIUM.—Spores germinated slowly. Fifteen weeks after they were sown, when growth was first discernible to the naked eye, the largest prothallia had just established a multicellular meristem. Their uniform growth was considered an indication that the cultures were pure; but the identity of young prothallia was also checked by the modeling of the spore coat, which could usually be found adhering to the basal end of the thallus.

Prothallia are of the usual delicate polypod type. Unicellular papillate hairs are found along the margins of young prothallia and scattered over the surfaces of mature ones. Hairs may arise superficially from any cell, frequently on an enlarged base and protruding from the thallus, especially in the case of marginal hairs (fig. 29).

Antheridia may arise directly from the surface of the thallus (figs. 27, 28), or they may be borne on a subglobose pedicel cell (fig. 26). In some cases two or even three antheridia of different ages arose from a single pedicel cell (fig. 26). The cell which in most polypods is designated as the "funnel cell" is more or less cylindrical rather than funnel-shaped (figs. 26, 27). When the antheridium opens, the undivided lid cell is extruded intact (fig. 28). Archegonia are of the usual polypod type.

Discussion

Acrophorus has been placed by some systematists among ferns of Davallioid or marginal derivation, by others among Cyatheoid or superficial derivatives. As pointed out by BOWER (2), its basket-like habit, its dictyostelic anatomy with a highly segregated leaf trace, and the presence of abundant chaffy scales indicate an affinity with *Dryopteris* and the superficial line.

The association of *Acrophorus* with the Marginales is based on two misconceptions: that the sorus is terminal on the vein and that the receptacle is marginal in origin. It has just been pointed out that the sorus is lateral rather than terminal on the veinlets; sections cut parallel to the vein show that a small extension from the veinlet enters the receptacle, where it ends as a slightly enlarged mass of tracheids (figs. 7, 8). Such an arrangement is unlike that in marginal derivatives, where the sorus, even when superficially placed at maturity, is located on a vein ending. Early stages in sorus development appear at first glance to have originated on the margin of the leaf, but the marginal initial itself is not involved in sorus formation.

Of the Superficiales, *Acrophorus* resembles both the Woodsioid and the

Dryopteroid groups. These two groups show a continuous progression in the development of a zygomorphic indusium, from *Woodsia* (11), with indusial hairs and scales which lag in development on the side toward the leaf margin, to *Peranema* (5), with a scale attached only on the side of the receptacle away from the leaf margin but completely inclosing the entire sorus, and to *Dryopteris*, with a basally attached indusium which has assumed a kidney shape related to the form of the expanded receptacle. *Acrophorus* has advanced with respect to its indusium to the same stage as *Peranema* and *Cystopteris*. The indusium in all three is attached along a broad base, free at the other margins and overarches the sorus completely.

The receptacle in all members of the two groups, including *Acrophorus*, is superficial in origin and situated near the end of a vein, but on a special short branch or extension rather than on the actual vein ending (figs. 6–8). The form of the receptacle is regularly round or hemispherical in the Woodsioideae, except in the case of *Peranema*, where it becomes tilted toward the leaf margin as it develops. In the Dryopteroids the receptacle is tilted from its first appearance and is overarched by the indusium before the first sporangium is initiated. Similar sections of very young fertile pinnules of *Dryopteris* and *Acrophorus* are much alike, except for the decided lag in the extension of the lamina in the latter.

Early stages in soral development of *Cystopteris* are strikingly similar to those of *Acrophorus*. Figures of *C. bulbifera* given by PALSER and BARRICK (10) might have been drawn from preparations of *Acrophorus*. *C. montana* (8) and *C. fragilis* (1) also closely resemble *Acrophorus* in the origin of the receptacle and indusium adjacent to but independent of the retarded leaf margin.

The Woodsioideae show transitions from gradate sporangial development in *Woodsia*, to gradate becoming mixed in *Peranema*, to purely mixed in *Hypoderris* and *Diacalpe*. *Dryopteris* shows gradate development at first but soon passes to the mixed condition. In this *Acrophorus* agrees with both *Peranema* and *Dryopteris* (2), as well as with *Cystopteris* (10). Sporangial segmentation in *Acrophorus* is like that in *Peranema*, in that the first wall dividing the initial cell may be either transverse or oblique (6). The transverse wall is characteristic of higher polypods, the oblique is characteristic of ferns on an evolutionary level with the Cyatheaceae. The mature sporangium is much like that of *Dryopteris filix-mas* in the number and arrangement of cells of the capsule, in the form of the stalk, and in the frequent occurrence of a hair on the stalk. In the presence of a perispore it agrees with most Dryopteroids and Woodsioids, although this structure is lacking in *Cystopteris* (9).

Of prothallial characters, those generally considered to be of phyletic significance are the nature of any hairs present and the condition of the antheridial lid cell. The unicellular hairs on the prothallium of *Acrophorus* closely resemble those described by DAVIE (6) for *Peranema* in their shape and distribution and in the fact that they are often borne on slightly elevated or protruding superficial cells. Such hairs are more like those of *Dryopteris* than those of the Woodsioideae. The antheridial lid cell is undivided, as it is in *W. ilvensis*, *Peranema*, *Cystopteris*, *Dryopteris*, and other higher polypods. The unindented funnel cell and the elevation of one or more antheridia on a globular pedicel cell seem to be somewhat unusual characters. Devel-

opmental stages of prothallia are similar to those figured by DOPP (7) for *Cystopteris fragilis*.

There is a strong resemblance between *Acrophorus* and *Cystopteris;* the chief differences are in size and habit and in the lack of a perispore in *Cystopteris*. It seems probable that these genera, while distinct, are very closely related.

CHRISTENSEN (4) places *Acrophorus* among a group of genera between the Woodsioideae and the Dryopteroideae. The structural details here described support this disposition. The zygomorphy of the sorus of *Acrophorus* from its first appearance and the early establishment of mixed sporangial sequence point to a close affinity with *Dryopteris*, although the unexpanded receptacle and the inconstant manner of segmentation of the sporangial initial indicate a less advanced state for *Acrophorus*.

Summary

1. The sorus of *Acrophorus stipellatus* is not terminal on a vein but is borne on a short branch arising near the vein ending.

2. The sorus originates close to the leaf margin, but the marginal initial cell is never involved in sorus construction. The sorus is zygomorphic from its first appearance. Sporangia arise at first in gradate but soon in mixed sequence.

3. The first segmentation of the sporangial initial may be either transverse or oblique. The sporangial stalk consists of three cell rows and sometimes bears a curved hair. The annulus is vertical and incomplete, with an unequal number of cells in the two faces of the capsule.

4. Prothallia are of the usual polypod type and bear unicellular papillate hairs. The antheridial "funnel cell" has the form of a solid cylinder. The lid cell is extruded intact at maturity.

5. The soral development and structure of *Acrophorus* bear striking resemblances to those of *Cystopteris*. *Acrophorus* appears to form a link between the more advanced of the Woodsioideae and *Dryopteris*.

The writer wishes to express sincere appreciation for the guidance and help of Professor ALMA G. STOKEY, who collected the material and under whose direction the work was carried out.

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ANATOMICAL STUDIES OF THE ROOTS OF JUVENILE LODGEPOLE PINE

RICHARD J. PRESTON, JR.

(WITH EIGHT FIGURES)

Introduction

The roots of forest trees, because of the difficulty and time involved in excavation, have received relatively little attention and study as compared with the above-ground parts. To a great extent the benefits which silvicultural operations have upon a forest result from changes brought about in the root systems, and a more thorough knowledge of the roots will place cutting, thinning, and planting practices on a sounder basis. This investigation was carried out in conjunction with an ecological study concerning the growth and development of root systems (**10**), in the hope that results might be attained which could be applied to the management of lodgepole pine.

METHODS.—The root systems of 105 specimens of lodgepole pine, *Pinus contorta* var. *latifolia* Engelm., which ranged from 1 to 15 years in age, were excavated during the summers of 1939 and 1940. These excavations were made on two different sites of lodgepole pine in the vicinity of the summer forestry camp of Colorado State College, at Pingree Park, Colorado. The roots were removed by using a pressure sprayer adjusted so as to eject a very fine stream of water. By this manner the root system could be uncovered with so slight damage that it was possible to count the number of mycorrhizae with considerable accuracy.

Anatomical studies of the different orders of roots from freshly excavated seedlings were made. Different techniques were used for the woody and nonwoody portions. The techniques, preserving fluids, and staining schedules used were those outlined by SASS (**11**).

The growing tips and mycorrhizae were killed and preserved in the Allen-Bouin type 2 formula. After dehydration, these nonwoody structures were infiltrated and imbedded in paraffin, mounted on wooden blocks, and sectioned at approximately 10 μ. They were stained with safranin and fast green. The woody parts were killed and preserved in formula FAA. This material, after dehydration, was infiltrated and imbedded in celloidin, mounted on wooden blocks, sectioned at about 15 μ, and stained with safranin and fast green.

Investigation

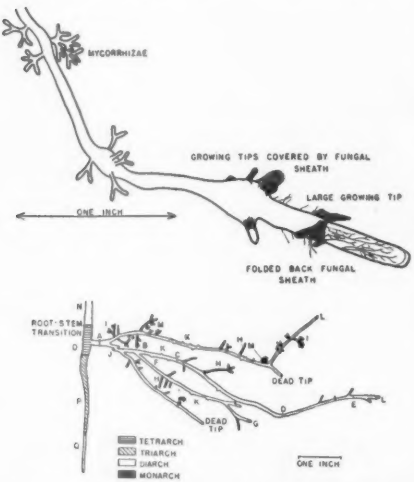
The root system of lodgepole pine can be classified into three main types, which differ in structure and development. These types are the radicle, primary, or taproot; the lateral long-roots; and the lateral short-roots. Lateral long-roots make up the more or less permanent lateral root system. Lateral short-roots differ structurally from long-roots and are temporary structures; in lodgepole pine they normally develop into mycorrhizae.

TAPROOT

The radicle normally developed into a rather deep taproot, which persisted throughout the life of the tree. If

through injury or other cause the taproot did not develop normally, its function was usually assumed by a lateral root, which grew vertically downward to a depth approximately that which the taproot would have attained.

During periods of active elongation, the growing tip became conspicuous, be-



FIGS. 1, 2.—Fig. 1 (above), sketch of root excavated April 13, 1940. Fig. 2 (below), portion of 2-year-old root system indicating stelar structure. Letters indicate points where sections were taken: *A*, *B*, *C*, *D*, *E*, *F*, lateral long-roots; *G*, large growing tip; *H*, long mycorrhiza; *I*, normal mycorrhiza; *J*, *K*, healings; *L*, small growing tip; *M*, ball mycorrhiza; *N*, stem immediately above root-stem transition; *O*, taproots (several) 0.5–1 inch deep; *P*, taproots (several) 1.5–2 inches deep; *Q*, taproots (several) 2.5–3 inches deep.

ing clear white and on some roots exceeding 2 inches in length (fig. 1). At these periods there was a well-developed root cap of oblong-shaped, thin-walled cells which had their long axes parallel to the axis of the root. The actively growing tips were commonly much thicker than the older portion of the roots, often having a diameter three times as great as the latter (fig. 1), and frequently attaining a diameter of $\frac{1}{8}$ inch. This thickened condition continued back for several inches on fast growing roots, until cork formation resulted in the shedding of cortical cells.

During periods of dormancy or semidormancy the root cap consisted of only a few cells, and the entire tip was often covered by a typical mycorrhizal sheath. When growth was renewed the tip burst through this sheath and again developed a large root cap (fig. 1).

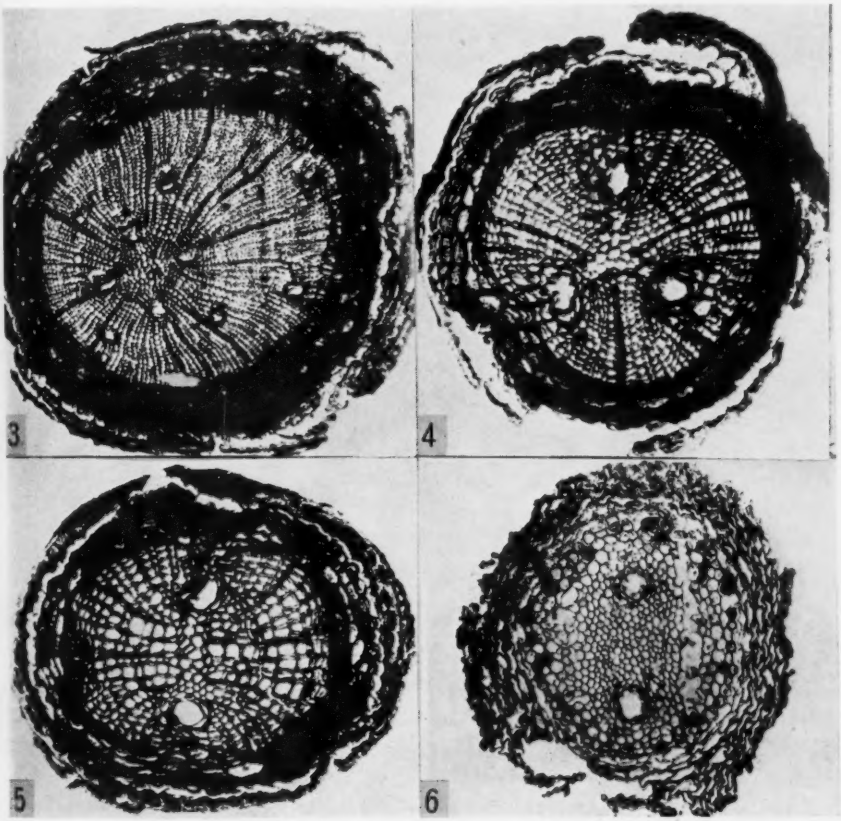
Root hairs developed a short distance behind the tip. According to LAING (5) and HATCH and DOAK (3), these are not epidermal but arise from the second or third layer of cortical cells. They disappeared shortly after the underlying cells became suberized. All actively growing taproots and long-roots had some root hairs, although never the abundant mat typical when grown on moist blotting paper.

Sections of four 2-year-old trees (fig. 2) were taken at distances from the root-stem transition of 0.5–1 inch, 1.5–2 inches, and 2.5–3 inches (hand-sectioning indicated that no structural changes occurred at greater distances). Stained sections through growing tips were likewise studied. No noteworthy differences from typical root development were noted in tissues outside the stele (2). The cortex, consisting of thin-walled, rather isodiametric cells with large intercellular spaces, varied from about four to seven layers in width. The endodermis, consisting of rectangular cells with the long axis arranged tangentially, was distinct.

The number of groups of primary xylem which developed was characteristic. The primary root (figs. 2, 3) was tetrarch for a distance of 0.5–1 inch immediately below the root-stem transition. From this distance to an average of 2.25 inches below it was triarch (fig. 4). Below 2.25 inches and for the remainder

of its development it was diarch (fig. 5). HATCH and DOAK (3) state that radicles are polyarch in *Pinus*, and ALDRICH-BLAKE (1) reports that most taproots are tetrarch throughout their length.

points of protoxylem matured at either side of each resin duct, and as differentiation continued centripetally a Y-shaped group of such cells developed on the side adjacent to each duct. These were con-



FIGS. 3–6.—Cross-sections: Fig. 3, taproot 0.5–1 inch below root-stem transition, tetrarch stele. Fig. 4, taproot 1.5–2 inches below root-stem transition, triarch stele. Fig. 5, taproot 2.5–3 inches below root-stem transition, diarch stele. Fig. 6, growing tip of lateral root, early diarch development.

The primary resin canals were differentiated very early in the development of the tissues (fig. 6) and were clearly evident before the endodermis became distinct. The epithelium of the resin ducts was thin-walled and surrounded by a second row of parenchyma. Two

nected by the differentiation of a bar across the center of the root. Eventually the remaining immature cells in the central portion of the root became differentiated into metaxylem (fig. 5).

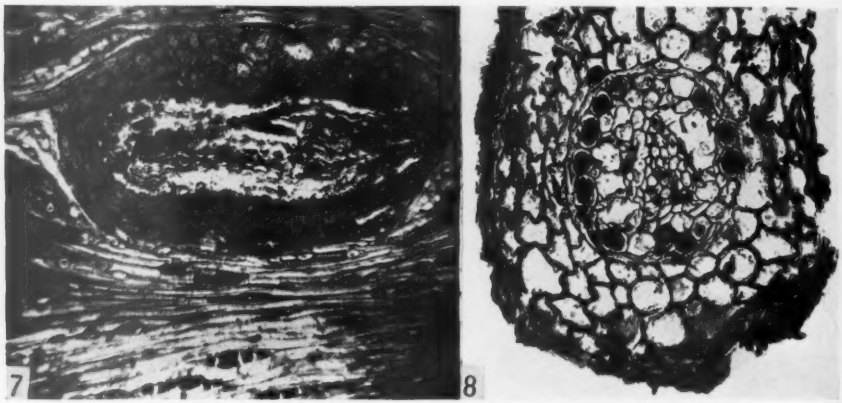
The majority of large secondary roots arose in the region immediately below

the root-stem transition. LIESE (6) concluded that in any particular species the greater the number of primary xylem bundles, the greater would be the abundance of rootlets and therefore the greater the potential absorptive surface of the juvenile root system.

LATERAL LONG-ROOTS

In all cases the lateral long-roots were diarch. There was no apparent difference in their structure from that of the diarch condition of the primary root. No differences were noted in six different lateral roots (fig. 2). HATCH and DOAK (3) state that in *Pinus* lateral long-roots are diarch in their proximal portion. These they term mother roots. More distally, however, they found many to be triarch and applied to these the term pioneer roots. ALDRICH-BLAKE (1) found this development of pioneer roots in Corsican pine but not in Scotch pine. In lodgepole pine lateral long-roots were diarch throughout.

Many diarch lateral roots abort before or during emergence from the parent root, or while still very short. Typical lateral roots show numerous small protuberances (fig. 2) which are either abortive roots or points where roots have died back to the parent root. Sections through these knobs showed that they had developed layers of cork cells over the portions exposed beyond the parent root. Other sections determined the type of healing that occurred when a root died back to a living root (fig. 7). In all cases cork cells were found separating the living from the dead tissue. VATER (12) states that when parts of the roots die off and disappear there is a periderm formed between the living and nonfunctioning portions.



FIGS. 7, 8.—Fig. 7, section through healing on lateral root, formation of periderm. Fig. 8, section through mycorrhizal short-root; monarch structure.

No diarch roots which had been attacked by a fungus and converted into a mycorrhiza (3) were found. HATCH (4) states that these are not common. Monarch short-roots were found which had grown to considerable length before being infected.

LATERAL SHORT-ROOTS

Lateral short-roots differ in several respects from the taproot and lateral long-roots. They are monarch (fig. 8), branch

dichotomously, and have no root caps. HATCH (4) states that mycrotrophy is the normal destiny of all short-roots, that they do not grow much in length, even if uninfected, and that they represent over 95 per cent of all roots in pine. These statements appear valid for lodgepole pine, as nearly all observed short-roots developed into typical ectotrophic mycorrhizae (10).

The mycorrhizal short-roots were not strictly annual structures. Roots excavated at the beginning of the growing season on April 13, 1940 (fig. 1), showed several mycorrhizae which were active, as evidenced by the white tips which had burst or split the fungal sheath. As these had developed the preceding year, this renewed activity rather definitely indicated that these particular roots were not annual. Further, no dead mycorrhizae were found on some vigorous roots several years old. This evidence is at variance with the conclusions of MCDOUGALL (9), MASUI (8), and ALDRICH-BLAKE (1), who state that mycorrhizae are annual. MCDOUGALL also concluded that the fungal sheath inhibited further growth of the roots converted into mycorrhizae, although MASUI (7) demonstrated that this was not necessarily the case, and that it was not uncommon for a mycorrhizal root to continue growth through the fungal sheath at the beginning of the growing season.

Summary

1. Studies of the root systems of juvenile lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) were made, excavations being carried out on two sites in Colorado. The entire root systems of 105 trees, ranging in age from 1 to 15 years, were excavated with a pressure sprayer.

2. Three main root types were recognized on the basis of structure and growth: the radicle, primary, or taproot; the lateral long-roots; and the lateral short-roots. Structural differences appeared in the stele. The taproot was found to be tetrach in its proximal portion, but more distally triarch and shortly thereafter diarch; all lateral long-roots were diarch throughout; and lateral short-roots were all monarch.

3. The taproot and lateral long-roots developed a typical root cap and root hairs and were capable of extensive growth. The lateral short-roots were not permanent structures, were restricted in growth, and almost invariably formed typical ectotrophic mycorrhizae.

4. The former place of attachment of roots which had died and disappeared, as well as the tips of aborted long-roots, developed a typical periderm which separated the living from the dead cells.

5. During the period of dormancy, mycorrhizal sheaths were found over the tips of lateral long-roots. Upon resumption of growth the tips burst through this sheath. No cases were noted where these long-roots had their growth permanently arrested and were converted into typical ectotrophic mycorrhizae by the fungus.

6. Mycorrhizae did not appear to be strictly annual. Several instances were noted where mycorrhizae had achieved renewed growth at the beginning of the growing season by bursting through the fungal sheath. Active mycorrhizae were also found on roots which were more than one year old.

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THE CHLOROPHYLL-PROTEIN COMPLEX. II. SPECIES RELATIONSHIPS IN CERTAIN LEGUMES AS SHOWN BY ELECTRIC MOBILITY CURVES[1]

LAURENCE S. MOYER AND MYER M. FISHMAN

(WITH FOUR FIGURES)

Introduction

Investigations of the specificity of plant proteins, either by means of serological, chemical, or physico-chemical methods, have usually had to deal with mixed systems. MEZ and colleagues (7), in establishing the "*phytoserologische Stammbaum*," generally used seed extracts for immunization. Quantity as well as quality could influence results obtained with these complex protein extracts and possibly lead to false conclusions. Thus absence of a cross reaction might simply indicate that one of the constituents was present at too low a concentration, rather than that an actual difference in quality was present. This complication has been recognized by MORITZ (8), who has developed anaphylactic methods for separation of the various components of the compound antigens; these permit a more complete analysis of serological relationship (9).

Physico-chemical as well as serological methods can be used for the characterization of proteins. Proteins adsorb either H^+ or OH^- and are therefore true amphoteric electrolytes. It has been shown (1) that when the sum of all the positive and negative charges on the surface of protein, over a time average, is equal to zero, its electrophoretic mobility is zero, and the protein or protein surface is isoelectric. Most proteins are isoelectric at acidities somewhat greater than neutral solutions, such that, at values of pH near neutrality, they are negatively charged. But as the pH is decreased, the electrophoretic mobility and net charge likewise decrease, becoming zero at the isoelectric point; below this point the surfaces become increasingly positive (1). The discovery (1) that the electrophoretic mobility of microscopic particles coated with adsorbed films of proteins is nearly or completely identical with that of dissolved protein molecules themselves made possible the investigation of native plant proteins by observation of the electrophoretic behavior of latex particles which are naturally coated with protein films or other ampholytes.

In the investigation of phylogenetic relationships in species of *Euphorbia* and *Asclepias* by the electrophoresis of their latex particles (10–12, 14, 15, 16), complexities similar to those discussed by MORITZ were frequently encountered. Although it was possible to characterize each species by its own electrophoretic mobility curve and isoelectric point, and to group these curves into classes of similar shape, which agreed with taxonomic relationships, nevertheless many species yielded complex curves. These suggested that not only proteins but also other substances with low isoelectric points, possibly sterols, were naturally present in the stabilizing film on the latex particle surface. Indeed, few of the curves had the smooth shape of electro-

[1] This work was materially aided by a grant from the funds of the Graduate School of the University of Minnesota.

phoretic mobility-pH curves of individual proteins (1). This diversity in the surface properties of the various latex particles was substantiated by an investigation of their wetting properties (10). Although phylogenetic relationship was clearly indicated by the results, it was realized at the time that changes in the relative amounts of the proteins and other ampholytes forming the surface films probably had as much to do with changes from species to species as had actual changes in the constitution of the proteins themselves.

Indeed, BONDY and FREUNDLICH (3) showed that the electric mobility curve for the latex of *Hevea brasiliensis* had a complex shape, closely similar to certain curves of latices from the Esulae section of *Euphorbia*, and that this was due to the interaction of two proteins which they isolated. In the pure state either protein exhibited a smooth electrophoretic curve, but when mixed in the presence of microscopic quartz particles, these became coated with a complex film and yielded a complicated curve very similar to that of the natural latex particles.

In view of these complications, it seemed desirable to compare the electrophoretic properties of a similar protein system occurring in a number of species. It would obviously not be significant to compare proteins in various species unless these were alike in function or origin. Inasmuch as it has been possible to isolate the chlorophyll-protein complex in a highly purified form from leaves of bean plants (4), preparation of the corresponding complex from a number of other legumes has been attempted. Since the chlorophyll-protein complex is a system with the same physiological function in these various species, with identical chlorophyll pigments, it seemed clear that the various complexes are legitimately comparable. LUBIMENKO (6) had observed that suspensions of the green material from different species of plants showed pronounced differences in properties. Some leaves gave clear, stable suspensions; some gave clear solutions which were unstable; others appeared to consist of chlorophyll tied to an insoluble protein. Because of the ready availability of young plants from various genera of the Leguminosae, attention was confined to this family for the present.

The literature on the electric mobilities and isoelectric points of other chromoproteins, from various animal species, has already been discussed (1). Some of the data were obtained at a single value of pH. Since such comparisons can lead to erroneous conclusions unless made over a wide range of pH (10), it was decided to determine the electric mobilities of the complex over the range of the acetate buffer system between pH 3.2 and 6.0.

Methods

Leaves from vigorously growing plants were ground in a mortar at 0° C. in the presence of M/100 phosphate buffer at pH 7.3–7.5. The resultant suspensions of the complex were purified by repeated precipitation with ammonium sulphate by the procedure of FISHMAN and MOYER (4). With some species the concentration of the ammonium sulphate was altered somewhat to produce a more clear-cut separation of the complex from the accompanying impurities. All preparations were dialyzed against M/100 phosphate buffers at 0° C. Since no preservatives were used, the electrophoretic measurements were made as soon as possible after purification. The electrophoretic techniques have been fully described elsewhere (1, 4). All measure-

ments were made at 25° C. in acetate buffers at an ionic strength of 0.02.

Results

The taxonomy in this paper follows that of BAILEY (2) and HEGI (5). All species investigated belonged to the section Papilionatae. With two exceptions the chlorophyll-protein complexes isolated from the various species were identical in general appearance. The dark green suspensions, although opaque, were quite stable. Attempts to prepare the complex from leaves of *Arachis hypogaea* produced a dark green suspension that blackened rapidly and became unstable. It seems likely that this anomalous behavior was due to contaminants not present in the other species. The complex from leaves of *Lupinus albus* became flocculated in the phosphate buffer and could not be resuspended. It is possible that its isoelectric point is so high that it is unstable at pH 7.3. These two genera belong to different tribes from those to which the rest of the genera belong: *Arachis* to the Coronilleae and *Lupinus* to the Genisteae. The other genera belong to (*a*) the Phaseoleae, (*b*) the Vicieae, or (*c*) the Trifolieae.

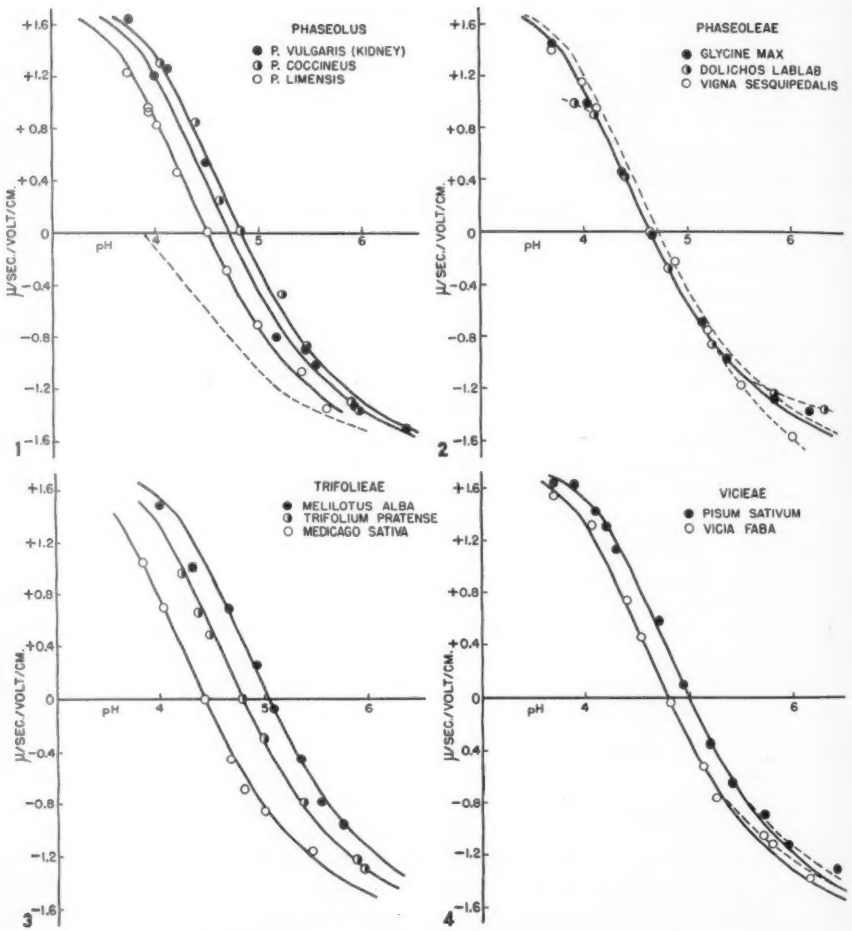
The electrophoretic curves were all regular in shape and characteristic in each case of a single protein. Each species could be characterized by a definite behavior. It soon became apparent that a single smooth curve would fit the data for any species, irrespective of the tribe to which it belonged. This fit resulted in each case when the curve was adjusted to the observed isoelectric point by shifting it to the left or right along the abscissa. Thus in figure 1, which represents the electric mobility-pH behavior of the chlorophyll-protein complex from species of *Phaseolus*, the central continuous curve (isoelectric at pH 4.70), which was drawn to fit the original data for navy bean (4), also describes the electrophoretic properties of the complex from *P. coccineus* and *P. limensis* when shifted to their respective isoelectric points. In fact, the original curve for navy bean fits the data for the closely related kidney bean without adjustment.

The curve of FISHMAN and MOYER for *Aspidistra elatior* is also plotted as a dashed line in figure 1. Since acid denaturation caused considerable shift and resultant discontinuity in the curve on the acid side of the isoelectric point, the curve has been ended at pH 3.9. Its slope and isoelectric point are completely different from the results on legumes.

In figure 2 are shown the results for *Vigna sesquipedalis*, *Dolichos lablab*, and *Glycine max*. In general, these fall along the same curve as in figure 1. Slight possible deviations have been indicated as dashed lines in the case of *Vigna* and *Dolichos*, but *Glycine* essentially follows the continuous curve. The complex from all three species appears to have the same isoelectric point. For comparison, a parallel curve (dashed) has been drawn to indicate the position of the data for *P. vulgaris*.

Figure 3 depicts the behavior of certain members of the Trifolieae: *Medicago sativa*, *Trifolium pratense*, and *Melilotus alba*. These results follow a course similar to the others.

The electric mobilities of the complex from *Vicia faba* and *Pisum sativum*, two genera of the Vicieae, are shown in figure 4. Here also the same curve has been superimposed on each set of data with equal agreement. A slight deviation appears to occur in each case. If significant, this common change in slope may serve to set the Vicieae apart from the other groups. Before this can be substantiated, more basic values of pH must be investi-



FIGS. 1–4.—Electrophoretic mobility–pH curves of chlorophyll-protein complex, in M/50 acetate buffer, at $\mu = 0.02$ and 25° C.: Fig. 1, kidney bean (solid circle), *P. coccineus* (half-solid circle), and *P. limensis* (open circle); smooth curves taken from data for navy bean, dashed curve from *Aspidistra elatior* (drawn for comparison). Fig. 2, *Glycine max* (solid circle), *Dolichos lablab* (half-solid circle), and *Vigna sesquipedalis* (open circle); smooth curve taken from data for navy bean (deviations indicated by dashed lines); parallel (dashed) curve is that of navy bean. Fig. 3, *Melilotus alba* (solid circle), *Trifolium pratense* (half-solid circle), and *Medicago sativa* (open circle); smooth curves taken from data for navy bean. Fig. 4, *Pisum sativum* (solid circle) and *Vicia faba* (open circle); smooth curves taken from data for navy bean (deviations indicated by dashed lines).

gated. To extend the curves to higher values of pH necessitates a change in the buffer system. Attempts to continue the curve for *P. vulgaris* by use of phosphate buffers (at the same ionic strength) led to an anomalous displacement in the curve, similar to that observed with rabbit serum proteins under comparable conditions (17). In view of this complication, the investigation of more basic regions has been deferred.

TABLE 1

ISOELECTRIC POINTS OF CHLOROPHYLL-PROTEIN COMPLEX FROM VARIOUS LEGUMES, AT 25° C. IN M/50 ACETATE BUFFERS

Species	Isoelectric point pH
Phaseoleae	
Phaseolus vulgaris var. navy bean	4.70
P. vulgaris var. kidney bean	4.70
P. limensis var. bush lima	4.50
P. coccineus	4.82
Vigna sesquipedalis	4.62
Dolichos lablab	4.62
Glycine max	4.62
Trifolieae	
Medicago sativa	4.42
Trifolium pratense	4.78
Melilotus alba	5.06
Viceae	
Vicia faba	4.78
Pisum sativum	4.96

TABLE 2

DATA FOR CONSTRUCTION OF TYPICAL CURVE FOR CHLOROPHYLL-PROTEIN COMPLEX FROM BEAN IN M/50 ACETATE BUFFERS AT 25° C.

pH	Electric mobility (μ/sec.)	pH	Electric mobility (μ/sec.)
3.5	+1.65	5.1	−0.59
3.7	+1.53	5.3	−0.83
3.9	+1.36	5.5	−1.02
4.1	+1.06	5.7	−1.17
4.3	+0.73	5.9	−1.31
4.5	+0.39	6.1	−1.41
4.7	0	6.3	−1.49
4.9	−0.30	6.5	−1.57

A list of isoelectric points has been assembled in table 1. Data for the construction of the characteristic smooth curve are given in table 2.

Discussion

At the start of these investigations, the close agreement found by SMITH (18) in the analyses of the chlorophyll-protein complexes from *Aspidistra* and *Spinacia* suggested that no essential differences would be found among genera. The marked divergences between the electrophoretic properties of the *Aspidistra* and bean preparations, however, indicated that clear differences did exist between widely separated genera (4). Within a homogeneous section like the Papilionatae there occurs less variation in physical characteristics of the complexes than exists between families and larger categories. Indeed, it is possible that in the Papilionatae the photosynthetic pigments are associated with essentially the same protein, which becomes only slightly modified from species to species.

Except in those cases in which divergences at the ends of the ideal curve have been suggested in the figures, the agreement of the data with the shifted curve is usually well within the limits of experimental error. As indicated, slight modifications do occur, but the general picture is essentially one of a single smooth curve, shifted up or down the pH scale but not changed in other features. A somewhat similar case was found with the latices from species of *Asclepias* (14). In that work it was suggested that the data could be fitted by a single curve, but that, owing to the narrow range of the buffer system, various parts of the whole curve fitted the results for each of the species investigated. It was as though the same general curve shape was being

retained, even though the isoelectric point changed from species to species.

Summary

1. The chlorophyll-protein complex has been prepared from leaves of the following legumes: *Phaseolus vulgaris, P. limensis, P. coccineus, Vigna sesquipedalis, Dolichos lablab, Glycine max, Medicago sativa, Trifolium pratense, Melilotus alba, Vicia faba,* and *Pisum sativum.*

2. Electrophoretic mobility-pH curves of the complex from these species show close relationship but differ completely from the curve for *Aspidistra.*

3. The various sets of data from these legumes could be described by a single smooth curve shifted up or down the pH scale to pass through each isoelectric point. In some cases minor deviations were noticed, but in general the agreement was good.

4. It is suggested that the complex from each species is characterized by essentially the same protein, slightly altered in certain respects from the rest but retaining enough of its original character to show relationship.

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EFFECTS OF VITAMINS ON GERMINATION AND GROWTH OF ORCHIDS[1]

G. R. NOGGLE AND F. L. WYND

Introduction

It is held by many students that the need for the symbiotic relationship between epiphytic orchids and certain fungi is eliminated if an appropriate supply of soluble carbohydrate be present in the substrate. The observation of many hundreds of orchid cultures, however, grown at the Missouri Botanical Garden, under many conditions of nutrition, shows that rarely do asymbiotic cultures thrive as well as those containing the appropriate symbiotic fungus. This has led to the assumption that the fungal symbiont was important for some reason in addition to that of hydrolyzing insoluble carbohydrates in the substrate so that they might be absorbed by its host.

The relation of the fungus to the acidity of the medium has been discussed by KNUDSON (13). The possible importance of the fungus in the nitrogen metabolism of the host has been suggested by others. TERNETZ (31) found that the fungal symbionts isolated from the roots of five species of the Ericaceae were able to fix atmospheric nitrogen in artificial cultures. DUGGAR and DAVIS (6) and RAYNER (22) also reported that nitrogen could be fixed by symbiotic fungi. The conclusions of RAYNER have been criticized on the basis that the amount of nitrogen fixed was so small that it was within the range of experimental error. WOLFF (34) believed that the endophyte of the orchid *Neottia* could fix atmospheric nitrogen, which was subsequently available to the host.

Recent investigations have emphasized the importance of certain vitamins and other growth factors for development of plants. It seems reasonable to suppose that the fungal symbiont might exert a favorable effect on the development of orchid seedlings by supplying some of these factors, in addition to hydrolyzing insoluble carbohydrates, and thereby account for their more rapid growth when cultured symbiotically.

An example of microörganisms supplying a growth factor to higher plants was reported by MCBURNEY, BOLLEN, and WILLIAMS (17), who found that the nodule-forming bacterium owes part of its stimulating effect to the pantothenic acid which was supplied to the host. MOCKERIDGE (18) showed that substances produced by bacteria increased the growth of *Lemna*. WILLIAMS and ROHRMAN (32) reported that the liverwort *Ricciocarpus* responded by marked increase in growth when pantothenic acid was added to the culture medium. ROBBINS (24) and MÜLLER and SCHOPFER (19) have suggested that symbiosis and parasitism might be intimately related to the ability of one member of the association to supply growth factors to the other.

BURGEFF (4) pointed out that certain physiologically highly specialized orchids of the *Vanda* group always developed slowly in the absence of their natural symbionts. The dead fungus was found to be just as effective as the living hyphae, because of the presence of a growth factor resembling "bios II," although its chemical nature was otherwise unknown.

More recently, WITHNER (33) cultured *Cattleya octave doin* × *C. mossiae* and *Epidendrum tempense* on media which contained various growth factors. He found that no effect was exerted by vitamin B_1, vitamin B_2, vitamin B_6, vitamin C, niacin, biotin, pantothenic acid, and

[1] The expenses incurred in this study were borne in part by a grant by the Cerophyll Laboratories, Inc., Kansas City, Missouri, to the Graduate School of the University of Illinois.

inositol. On the other hand, POLLACCI and BERGANISCHI (**20**) found that the presence of vitamin C in the medium resulted in increased germination and accelerated the growth of *Cattleya labiata autumnalis* and *Oncidium pulvinatum*.

The necessity of an external supply of various growth factors for higher plants has been reported. The importance of vitamin B_1 has been shown (**14, 3, 27**). The influence of vitamin C in germination and growth has also been recorded (**9, 3, 10**). The importance of vitamin B_2 in the nutrient medium for the growth of higher plants has not yet been conclusively demonstrated. The present study sought additional information on the effect of certain vitamins on the germination of the seeds and on the growth of orchids.

Investigation

EXPERIMENT I

A mature seedpod of the hybrid, *Cattleya trianae* var. *mooreana* × *C. schroederae*, was obtained through the generosity of DR. GEORGE T. MOORE, Director of the Missouri Botanical Garden. It had been picked in January, 1940, and had been kept in a refrigerator at 7° C. until the seeds were sown, February 5, 1941. The nutrient solution was the type I, R_1S_2, described by LIVINGSTON (**16**), which had produced excellent germination and growth during earlier experiments (**35, 36**). In addition to the six major nutrient ions, iron, manganese, and boron were added as formerly, and the pH was adjusted to 5.0. The carbohydrate was supplied as 1.5 per cent maltose. Solidification was obtained by 2 per cent Difco agar. (The carbohydrate was a technical grade of maltose, manufactured by Daigger and Company. Its light brown color and gummy texture indicated that the material had not been highly refined.) Ten flasks were maintained as a control series; ten received 0.1 mg. per liter of thiamin hydrochloride, ten received 0.01 mg., and ten received 0.005 mg. per liter of this vitamin.

Good germination and growth occurred when these nutrient media were used. The addition of thiamin hydrochloride was without effect. On October 7, 1941, 8 months later, the seedlings were transplanted by means of an inoculating needle to flasks of media freshly prepared as before, except that Pfansteil's reagent maltose was substituted for the older, less refined sugar. Some of these cultures received 10 mg. per liter of ascorbic acid (vitamin C), others received 1 mg. per liter, while others served as control cultures. Although sixty flasks of transplanted seedlings composed this series, in every flask the seedlings turned yellow in a few weeks and finally became almost white. Significant growth did not occur, and all seedlings died within 3 months. Tests made on the media showed that the proper acidity had been maintained. There was no effect due to any concentration of ascorbic acid.

EXPERIMENT II

Another pod of *Cattleya* hybrid which showed a remarkably high percentage of fertile seeds was obtained from the Missouri Botanical Gardens. These seeds were inoculated into series of media prepared as in experiment I, except that the thiamin hydrochloride was added in a greater series of concentrations (10.0, 5.0, 1.0, 0.5, 0.1, 0.05, 0.01, and 0.001 mg. per liter). The carbohydrate, unlike that of experiment I, was the highly purified reagent grade of maltose prepared by the Pfansteil Company.

After 6 weeks the protocorms were still so small that they could scarcely be seen with the unaided eye. After several months they had increased materially in size but had become a shapeless structure

of parenchymatous tissue. Normal leaves did not appear, and chlorophyll was absent—or present only in traces. The physical conditions had been ideal, and tests showed that the proper acidity had been maintained. There was no effect from the addition of thiamin hydrochloride.

EXPERIMENT III

The results of experiments I and II indicated that the impure technical grade of maltose contained some factor or factors necessary for the germination and growth of orchid seedlings which were not present in the particular sample of purified maltose. It also appeared that neither ascorbic acid nor thiamin hydrochloride could replace these substances. In the meantime, a third experiment had been started, using the seeds from the same pod as those used in experiment II. The media were prepared with the reagent grade of maltose as the carbohydrate. Riboflavin (vitamin B_2), nicotinic acid (P-P factor), pyridoxine hydrochloride (vitamin B_6), and calcium pantothenate (chick antidermatitis factor) were added, each to separate cultures, in concentrations of 10 and 1 mg. per liter. After 14 months the following observations were made:

CONTROL.—These cultures showed practically no germination. Not more than a dozen seeds out of many hundreds formed protocorms, and these remained small, distorted in shape, and without chlorophyll. All were dead within a few months.

CALCIUM PANTOTHENATE.—No significant germination occurred.

RIBOFLAVIN.—Very limited germination occurred. The protocorms averaged 0.88 mm. in diameter, and they had developed one to three normally shaped but very small green leaves. Germination was so poor and subsequent growth so limited that the beneficial effect of this vitamin appears doubtful. The seedlings did develop a green color, however, which was not the case for the controls.

PYRIDOXINE.—Excellent germination occurred, but subsequent development was slow. The protocorms became abnormally large and were frequently distorted in shape. The protocorms averaged 1.12 mm. in diameter. A few had developed one or two rudimentary leaves, although most of them exhibited only one or two undeveloped leaf points. The average height was 2.40 mm. Chlorophyll development was conspicuously less than normal. Perhaps one-half the protocorms developed a pale green color, but the others remained with little or no chlorophyll.

NICOTINIC ACID.—This series produced excellent germination, and subsequent development was good. The protocorms remained small, and normal green leaves developed. The concentration of 1 mg. per liter produced the most rapid growth of the seedlings. In this concentration the protocorms averaged 0.86 mm. in diameter and the seedlings averaged 4.40 mm. in height. The average number of well-developed leaves was 3.16. This was the only medium in which the seedlings developed aerial roots, some of which attained a length of 6 mm. The concentration of 10 mg. per liter produced protocorms which averaged 0.75 mm. in diameter and seedlings which averaged 3.44 mm. in height, while the number of normal leaves per plant averaged 2.40.

Although the seedlings in these cultures appeared to have normal shape and color, their growth was far less than that of normal seedlings of an equal age. Under the most favorable conditions, orchid seedlings should be about $\frac{1}{4}$ inch high in 4 months. At 10–12 months they should be large enough to be transplanted. Apparently there are other factors or

combinations of factors necessary for the normal growth of orchids besides those described here.

Instances in which erratic results in various types of culture experiments were traceable to biologically active impurities in the reagents are not infrequent. The probability that commercially prepared sugars vary significantly in their freedom from effective growth factors may account for the divergent views of various students concerning the most favorable type of sugar for orchid germination and growth. LA GARDE (15) stated that maltose was the best sugar for the growth of orchids, QUEDNOW (21) found d-glucose the best, while WYND (36) found d-mannose to give the best growth. WITHNER (33) reported that sugar has been shown to contain small amounts of vitamin B_1, vitamin B_6, biotin, and probably other substances.

The carbohydrates are not the only sources which might supply growth factors to cultures as impurities. Agar contains variable amounts of impurities, even when it is highly purified. ROBBINS and WHITE (29) showed that some of these impurities were biologically effective, since a water extract of agar increased the growth rate of excised corn roots. ROBBINS (23) later reported that Difco agar exerted a beneficial effect on the germination of spores, growth, and the formation of gametes and zygotes by *Phycomyces blakesleeanus*. The stimulating effect was believed to be due to unrecognized growth factors. DAY (5) found that *P. blakesleeanus* grew better when Difco or Eimer & Amend agar was added to the medium. Since no thiamin had been added, she concluded that this necessary substance was introduced as an impurity of the agar. ALLISON and HOOVER (1) demonstrated the presence of coenzyme R (=biotin or vitamin H) in agar. HAWKER (11, 12) found biotin to be present in agar. Thiamin has been found in agar by FRIES (7) and FROMAGEOT and TCHANG (8). Unidentified growth substances, called factor Z, were found in agar by ROBBINS (25). ROBBINS (26) and ROBBINS and MA (28) found significant amounts of biotin in some samples of agar as determined by the growth of *Ashbya gossypii*. SCHOPFER and RYTZ (30) found that crude cotton contained thiamin or its intermediate products. DAY (5) found that cheesecloth and filter paper also contained sufficient thiamin to affect the growth of *Phycomyces*.

In view of the ever-present possibility of introducing growth substances into culture media by impure reagents or contact with material used in the laboratory, different workers may not obtain similar results in this field of research unless extremely careful precautions are taken in laboratory procedures.

Summary

1. Seeds of the orchid *Cattleya trianae* var. *mooreana* × *C. schroederae* germinated and produced normal growth in an artificial nutrient medium in which one lot of maltose was used as a source of carbohydrate, but no germination of seeds or growth of seedlings was obtained when a more purified maltose was used.

2. The inability of the orchid seeds to germinate and to develop on the purified maltose was not overcome by the addition of thiamin hydrochloride (vitamin B_1), ascorbic acid (vitamin C), or calcium pantothenate (chick antidermatitis factor).

3. A few seeds germinated and slow development of the seedlings occurred when riboflavin (vitamin B_2) was present in the medium.

4. The presence of pyridoxine (vita-

min B_6) permitted good germination, but subsequent development was poor.

5. Good germination and excellent development of the seedlings occurred when nicotinic acid (P-P factor) was supplied in the nutrient medium.

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TRANSPLANTATION EXPERIMENTS IN PEAS. III[1]

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Introduction

Earlier papers (**12, 5**) have shown that a number of specific growth factors can be transported across the graft union of pea seedlings as soon as vascular connections between the graft partners have been established. By choosing the proper combinations, either the growth-factor content of the stock or the growth response of the scion can be studied. In this way a tool is provided for the investigation of growth factors which cannot be extracted as yet. Although this type of experiment has yielded many important results in embryonic development of animals, and also has been shown adaptable to plants, very little work has been published on the graft technique for growth-factor analysis. The most important contribution in this field is that of WETTSTEIN and PIRSCHLE (**11**) concerning a gene-determined factor in *Petunia* transmitted by grafting.

The conditions under which the experiments were carried out are almost identical with those prevailing in the earlier experiments of this series (**12, 5**). From soaking until termination of the experiment, the peas are kept in physiological darkrooms, maintained at 24° C. and 80 per cent humidity. An orange light is kept on for 4–8 hours each day in the rooms, providing enough light for leaf and stipule development (**14**).

Some varieties of pea have to be disinfected before planting by washing them for 1 minute in 90 per cent alcohol, followed by 20 minutes in 0.1 per cent $HgCl_2$, and repeated washing with sterile water. Then they are soaked for 5 hours in water and spread out on moist filter paper in dishes. As soon as the roots have reached a length of 1–2 cm., the peas are transferred singly to 20-cc. wide-neck bottles or glass tubes. The seed coats have to be removed to prevent growth of molds and bacteria. A few drops of water are added, enough to provide for 1 day's growth. Each day the required amount of water is supplied, but not enough to submerge the roots (this would greatly decrease the growth rate of the shoots). Six to 8 days after soaking (or longer for a few slow-growing varieties), the peas are grafted by cutting the stem at a 30° angle and slipping the two cut ends through a short glass tube with approximately the same bore as the stem diameter. From then on, daily measurements are taken to determine maximal growth rates.

The grafted peas are inspected daily, and lateral buds developing on the stock are removed before they can inhibit growth of the scion. Throughout this period the root system is kept moist but never submerged in water.

When growth in length of the main stem of the scion has stopped, lateral buds will develop. These buds soon grow 50 per cent more rapidly than the main shoot. For this reason, growth rates of laterals and main stem cannot be averaged. At the end of the growth period the length of the leaflet, stipule, petiole, and tendril of each successive leaf on the scion is measured. In general, the oldest leaflet is already almost full-grown at the time of grafting, so that it

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does not show a clear stock effect; but the dimensions of the younger leaves can be used for calculations of the stock-scion relationship.

The present paper describes some further experiments concerning the technique of grafting, some experiments concerning the nature of the stock-scion relation, and also a few applications of the technique.

shorter than in the regular grafting experiments. The 8-day-old peas were 130–200 mm. long. The 10-day-old plants were 250–300 mm. long—much longer than otherwise used for grafting. The results as presented in table 1 can be summarized as follows:

Six- and 8-day-old peas, within a range of 50–200 mm. length, are equally suitable for grafting, both as stock and as

TABLE 1

EFFECT OF AGE OF ROOT STOCK AND SCION OF ALASKA PEAS AT TIME OF GRAFTING: ON PERCENTAGE OF SUCCESSFUL GRAFTS (EACH GROUP CONSISTING OF ABOUT FIFTEEN), ON MAXIMAL GROWTH RATE OF SCIONS AFTER GRAFTING, ON LENGTH OF SUM OF SECOND AND THIRD INTERNODES OF SCION, ON LEAF, AND ON STIPULE SURFACE OF SCIONS (TAKEN AS MEAN OF SECOND AND THIRD LEAVES)

Stock	Scion											
	Ten days old		Eight days old					Six days old				
	Percentage take	Growth rate (mm./day)	Percentage take	Growth rate (mm./day)	Length of second and third internodes (mm.)	Leaf surface (mm.²)	Stipule surface (mm.²)	Percentage take	Growth rate (mm./day)	Length of second and third internodes (mm.)	Leaf surface (mm.²)	Stipule surface (mm.²)
Ten days old.....	20	19	21	17	75	32	46	67	25	87	19	25
Eight days old.....	21	20	62	32	125	42	48	56	33	139	32	40
Six days old.....	7	.....	69	33	140	58	54	77	31	139	28	36

Experimentation

Age of stock and scion at time of grafting

It had already been noted that when peas are left too long growing in darkness, the percentage of successful grafts with the seedlings is very low. Alaska peas were soaked and planted in glass bottles at 2-day intervals. When the different groups were 6, 8, and 10 days old, all possible graft combinations among the three groups were made. The 6-day-old plants were 50–100 mm. long—scion. The 10-day-old plants are very poor for grafting. This holds only for peas grown in a darkroom, however, when no assimilation occurs and the cotyledons are becoming exhausted.

Treatment of graft surface with hormones

In some preliminary experiments, Dr. A. C. Sinha, working in this laboratory, had observed certain effects of the treatment of the cut surface of pea seedlings just prior to grafting. Lanolin, either plain or mixed with various con-

centrations of indoleacetic acid, was injurious and killed the stems adjoining the graft in a few days, without any indication of junction of stock and scion. Dipping the cut ends of stock and scion for a few seconds in aqueous solutions of either indoleacetic and traumatic acids (100 p.p.m.) seemed to improve the taking of the grafts. This has been investigated in greater detail.

Peas, 7–8 days after germination, were grafted on themselves after removal of an intermediate stem piece approximately 50–100 mm. long. Before pressing the cut surfaces together in the glass tube, they were dipped for 1–2 seconds in various solutions. Afterward the growth measurements were taken in the ordinary way. Table 2 shows the results. Just 50 per cent of all grafts (forty per group) were successful. There was no preference for any treatment. The maximal growth rates, however, which were slightly higher than in most experiments, were all within the limits of experimental error. The same can be said of the leaf sizes of the scions and of the time necessary to reach maximal growth rate.

Another experiment indicated some improvement in maximal growth rate and percentage of successful cuttings when the cut ends are treated with a lower concentration (50 p.p.m.) of indoleacetic acid, but these increases do not warrant a change in the experimental procedure of grafting.

An investigation of the correlation between the length of the glass collar around the graft union and the growth of the scion showed that the mean length of the tube in 162 unsuccessful grafts was 7.95 mm., and in 200 successful grafts it was 7.95 mm. (the actual lengths varied between 4 and 12 mm.). In the successful grafts there was a slight preponderance (47 per cent) of the collars of 7 and 8 mm. length, compared with the unsuccessful grafts (only 36 per cent of the collars having a length of 7–8 mm.).

TABLE 2

MAXIMAL GROWTH RATES AND LEAF DEVELOPMENT OF SCIONS OF ALASKA PEAS, GRAFTED ON ALASKA STOCKS AFTER IMMERSION OF CUT SURFACES IN SOLUTIONS. FORTY GRAFTS IN EACH GROUP

CUT ENDS OF STOCK AND SCION WASHED WITH	PERCENTAGE SUCCESSFUL GRAFTS	MAXIMAL GROWTH RATE		LEAF LENGTH (MM.)		
		Rate (mm./day)	Reached after (days)	First leaf	Second leaf	Third leaf
Distilled water	52	46.2	7.5	5.8	6.3	7.0
Thiamin (1 p.p.m.)	45	42.9	7.8	5.6	6.1	7.0
Indoleacetic acid (50 p.p.m.)	45	47.7	8.0	5.2	6.7	6.7
Traumatic acid (100 p.p.m.)	55	46.3	7.6	5.6	6.7	7.0
Thiamin (0.67 p.p.m.), indoleacetic acid (33 p.p.m.), traumatic acid (67 p.p.m.)	52	47.3	7.4	5.8	6.6	7.5

EFFECT OF INTERMEDIATE STEM PIECE ON GROWTH OF SCION

ROBERTS (6) has attributed the differences in growth rate of scions on different stocks to properties of the stem of the stock rather than to the root system. This is evidenced by certain double-worked apple trees, where the scion acquires the growth habit of the intermediate piece of grafted stem. Since it is

possible to double-graft etiolated peas (5), intermediate stem pieces of different varieties were grafted, and the effects on leaf, stipule, and stem growth of scion were measured.

Four experiments, totaling 826 double-grafted peas, were carried out to determine the interstock effect. Only 9 per cent of the total number of double grafts took, but the results obtained with these 76 successful grafts are sufficient to indicate that an intermediate stem piece about 50 mm. long does not affect scion development to any measurable extent. In one experiment, stock was Little Marvel and scion Alaska; in two others both stock and scion were Alaska; and in the last experiment Stratagem was used as stock and scion. There were no systematic differences in the development of the scion in these four combinations when different interstocks were grafted between them, so that the results are combined in table 3. To make all data comparable, the effect of Alaska as interstock has been taken as 100, so that the effects of Perfection, Stratagem, and Little Marvel are expressed in percentages of Alaska, a procedure previously followed (12). The data for comparison of these effects with those of the same varieties used as stock were obtained by combining data of this paper with those of the previous one.

Whereas Little Marvel—and especially Perfection—reduces stem growth when used as stock, they do not do so at all when used as interstock. When Alaska, Stratagem, Little Marvel, and Perfection are used as intermediate pieces, the differences in stem growth of scion are not great enough to indicate that any effect exists. The same can be said for leaf and stipule growth. But Stratagem and Little Marvel, when used as stock, definitely increase leaf growth.

If the intermediate stem piece were of any importance in determining the rate of development of the scion, it would make a difference whether a long scion were grafted on a short stock or a short scion on a long stock. Alaska peas were used as stock and Little Marvel as scion. One group had a 14-mm. stock and a 70-mm. scion; the other group had a 65-mm. stock and a 20-mm. scion. In both groups nine grafts took out of twenty; maximal growth rates were 14.4 ± 1.0 mm./day for long and 14.0 ± 0.6 mm./day for short scions. Leaf and stipule

TABLE 3

GROWTH OF PEA SEEDLING SCIONS EITHER GRAFTED ON VARIETIES MENTIONED OR ON SAME STOCK BUT WITH INTERMEDIATE STEM PIECE (DOUBLE-GRAFT). DATA FOR STOCK EFFECTS BASED ON ALL PEA-GRAFTING EXPERIMENTS TO DATE

STOCK OR INTERSTOCK	GROWTH IN LENGTH OF SCION EXPRESSED AS PERCENTAGE OF EFFECT OF ALASKA								SUCCESSFUL DOUBLE GRAFTS
	Stock (single grafts, data from this paper and 12)				Interstock (data from 826 double-grafted peas)				
	Stem	Leaf	Stipule	Petiole	Stem	Leaf	Stipule	Petiole	
Alaska	100	100	100	100	100 ± 3	100 ± 5	100	100	18
Stratagem	95	129	121	114	102 ± 6	105 ± 6	100	100	26
Little Marvel	84	122	103	105	108	108	.....	.....	7
Perfection	63	109	103	105	109 ± 6	100 ± 5	97	109	13

surfaces of second, third, and fourth leaves combined were 50 and 33 mm.² for long and 52 and 38 mm.² for short scions, showing that under these conditions also no effect of an intermediate piece was found on the scion.

If these experiments are considered together, they indicate that there is no systematic difference in the growth of the scion when different varieties are used as intermediate stem pieces. This means that varieties which as stock greatly influence leaf growth or growth in length of the scion do not do so by means of the character of their stem or by the type of graft union. Only their root system and cotyledons can be responsible for the development of the scion. This is the same conclusion as reached in the earlier papers, on different grounds, and of course holds only for these etiolated peas, where the growth rate of the scion is limited by the supply of growth factors from the seed and not by the rate of translocation through stem or graft union, as seems to be the case in double-worked apple trees.

Correlation between auxin content, growth rate, and nature of stock and scion

Two extensive grafting experiments, one comprising 560 grafts and the other 400, were carried out to determine the correlation of auxin content with growth rate. When the scions were growing rapidly, their 15-mm. long tips were cut off and placed in redistilled ether. At least five tips had to be extracted simultaneously to obtain a significant amount of auxin. The extract was taken up in 0.3, 0.5, or 1.0 cc. of 1.5 per cent agar and poured into two sets of twelve blocks, so that each extraction was assayed on twenty-four test plants.

In the first of these experiments, stocks of Alaska, Daisy, Little Marvel, Morse's 200, and Perfection were grafted with scions of either Alaska, Daisy, or Little Marvel. Forty grafts of each combination were made, and in the different groups 22–70 per cent of all grafts succeeded, with an overall efficiency of 36 per cent (table 4). The auxin content of the tips is given as indoleacetic-acid equivalents (10), which means that such concentration of the acid, if present in the tips, would have given the same curvature as that observed. At the time of extraction not all scions had the same growth rate, and this might affect the results. For this reason in most cases the tips were divided into two or three groups, with high, medium, and low growth rates. But no significant correlation between auxin content and growth rate was found. The auxin content is not related to the stock variety, to the growth rate, or to the scion variety. Since there are strict correlations between stock and scion variety on the one hand and growth rate on the other, it is evident that auxin is not limiting the growth rate in these scions. This confirms the earlier conclusion (12) that the growth rate of the scions is determined by a growth factor other than auxin, coming from the stock.

In table 4 the data for stem growth, leaf, stipule, petiole, and tendril development in general confirm those already presented (12). There is one general difference: the Alaska stocks give a relatively greater leaf and stipule growth compared with the other root stocks. This might be attributed to the fact that all the other stocks are from the same seeds as used in the former paper, whereas the Alaska stocks come from a new batch of seeds, of a harvest one or two years later.

Daisy as root stock produces in 95 per

TABLE 4

PEA SEEDLING SCIONS GRAFTED APRIL 19–21, 1938, AND MEASURED 2 WEEKS LATER. FORTY SEEDLINGS GRAFTED PER GROUP

Root stock	Scion	Percentage successful grafts	Maximal growth rate (mm./day)	Auxin content of stem tips*	Second leaf				Third leaf				Fourth leaf			
					Leaf surface ($mm.^2$)	Stipule surface ($mm.^2$)	Petiole length (mm.)	Tendril length (mm.)	Leaf surface ($mm.^2$)	Stipule surface ($mm.^2$)	Petiole length (mm.)	Tendril length (mm.)	Leaf surface ($mm.^2$)	Stipule surface ($mm.^2$)	Petiole length (mm.)	Tendril length (mm.)
Daisy	Alaska	25	38.7±5.3	36	46	33	6.2	4.8	52	37	13.7	8.0	.....	.....	.....	.....
Morse's 200	Alaska	22	25.4±2.0	44	22	22	4.3	3.2	27	24	7.7	7.0	.....	.....	.....	.....
Alaska	Alaska	25	37.7±1.8	32	30	28	4.7	3.8	36	26	12.3	7.9	.....	.....	.....	.....
Little Marvel	Alaska	25	35.7±3.7	45	50	35	6.2	4.7	50	36	8.8	7.6	.....	.....	.....	.....
Perfection	Alaska	22	17.6±0.6	34	21	19	4.0	3.4	16	16	3.5	4.2	.....	.....	.....	.....
Daisy	Daisy	35	10.4±0.7	36	72	42	5.1	3.2	103	83	8.8	5.5	100	63	9.2	11.2
Morse's 200	Daisy	60	11.0±0.6	5	50	35	5.0	2.6	77	53	7.5	5.4	61	56	10.0	9.5
Alaska	Daisy	70	10.8±0.4	24	44	34	4.4	3.3	61	39	6.0	5.6	66	43	8.4	10.4
Little Marvel	Daisy	32	8.1±0.5	17	69	46	5.4	3.8	61	40	5.8	5.1	74	33	7.6	10.3
Perfection	Daisy	32	6.2±0.6	28	29	25	3.7	2.7	37	20	4.0	4.8	.....	.....	.....	.....
Daisy	Little Marvel	35	9.9±0.8	35	.....	.....	.....	.....	69	64	8.8	8.9	121	75	11.8	14.6
Morse's 200	Little Marvel	50	10.0±0.6	.....	.....	.....	.....	.....	82	56	9.2	9.6	.....	.....	.....	.....
Alaska	Little Marvel	40	9.5±0.4	32	.....	.....	.....	.....	72	51	7.5	7.4	91	50	10.4	12.9
Little Marvel	Little Marvel	30	8.2±0.5	7	.....	.....	.....	.....	50	44	6.1	8.1	77	38	8.1	8.7

* In 10^{-4} gamma/kg. indoleacetic-acid equivalents.

cent of the cases larger leaves and stipules and longer petioles than when Alaska is used as root stock, although the stem growth rate of scions is the same for both root stocks. Little Marvel and Perfection both decrease growth in length, compared with Alaska stocks.

Since this experiment might be taken as an indication that upon storage the growth-factor content of pea seeds decreases, seed samples of the same lines but of different ages were used (obtained through the courtesy of the Ferry-Morse Seed Company). The seeds were superficially sterilized and after 6 hours' soaking in water were laid out on moist filter paper. Especially of the older seeds, a considerable percentage rotted before germination; in some instances no seedlings were obtained at all. After germination, the seeds were transferred to 20-cc. bottles with a small amount of water, and the growth rate was measured before grafting (table 5). The grafting was carried out in the regular manner, and maximal growth rate and leaf, stipule, petiole, and tendril size were determined in the scions of the successful grafts. In this experiment the auxin content is expressed in 10^{-4} gamma indoleacetic-acid equivalents per tip, the method of extraction and determination being the same as in the previous experiment (table 4).

The most important results indicated in table 5 are the following: (*a*) There is no consistent decrease in either growth rate or leaf development of scions grafted on older seeds, nor does the percentage of successful grafts fall off. In some cases less auxin is extracted from such scions, but in others not. (*b*) With aging, the percentage of rotting seeds (which are superficially sterilized) rapidly increases, but there is no indication of a decreasing growth-factor content of the seeds involved. The shoots which developed on the older seeds were perfectly normal, but owing to early infection most of them never grew at all. (*c*) There is no correlation between maximal growth rate of the scion and its auxin content.

Data on root formation are included in table 5. The successful scions were cut in sections 50–80 mm. long and placed overnight with their base in 200 mg./l. indoleacetic acid. For the next four days they stood in 2 per cent sucrose, and after that in 2 per cent sucrose with 0.01 mg./l. thiamin added. Alaska stems formed on an average more roots per cutting than did Daisy stems, but there is no obvious correlation between rooting and root stock. The data were also analyzed in a different way. Earlier experiments have shown (13) that in addition to a number of chemically identified factors, root formation in pea stems requires an unknown factor, rhizocaline, which is supplied from the cotyledons and which gradually disappears when the connection between stem and cotyledon is severed. Which relation exists between the taking of the graft and the rooting response of the scion? Very few stems rooted which had only recently started to grow and had not yet reached their maximal growth rate. The best rooting was obtained on the scions growing at their maximal rate. When the growth rate of scion was decreasing again, owing to either exhaustion of stock or breakdown of the graft union (evidenced by brown decoloration), rooting also became less abundant. This indicates that rhizocaline also moves across a graft union, perhaps slightly lagging behind caulocaline, and that a continuous supply from the stock is needed to keep up the ability of scions to root.

Nitrogen metabolism of graft partners

Determinations of the nitrogen fractions of scions were carried out by Dr.

TABLE 5

PEA SEEDLING SCIONS GRAFTED DECEMBER, 1938, AND MEASURED 2 WEEKS LATER. FORTY SEEDLINGS GRAFTED PER GROUP

Stock	Scion	Growth rate of stock before grafting	Stocks rotted before grafting (%)	No. of scions growing	Percentage successful grafts	Maximal growth rate of scion	Auxin content of scion per tip*	Root formation on scions	Second leaf surface (mm.²)	Second stipule surface (mm.²)	Second petiole length (mm.)	Second tendril length (mm.)	Third leaf surface (mm.²)	Third stipule surface (mm.²)	Third petiole length (mm.)	Third tendril length (mm.)
Alaska 1937.....	Daisy	18.4	2	25	66	15.4	7.5	1.7	57	32	5.3	3.3	63	41	6.0	4.8
Alaska 1936.....	Daisy	18.0	0	23	60	16.8	11.5	1.9	43	26	4.9	3.3	59	36	6.3	4.7
Alaska 1935.....	Daisy	19.0	3	15	39	17.1	8.3	0.9	35	27	4.5	3.3	44	30	6.3	5.0
Hundredfold 1937..	Daisy	2.1	49	3	23	13	.....	.....	90	69	7.7	4.7	135	101	11.7	9.0
Hundredfold 1936..	Daisy	3.3	37	3	21	11	.....	.....	115	80	8.2	7.2	173	48	11.0	10.0
Hundredfold 1935..	Daisy	2.8	50	4	25	.....	.....	.....	92	62	6.5	3.2	116	75	12.0	7.0
Stratagem 1938....	Daisy	9.8	17	15	42	15.9	32.1	0.6	70	40	6.0	3.6	85	54	8.4	6.3
Stratagem 1936....	Daisy	8.0	23	12	40	13.5	19.7	0.8	83	54	5.9	4.4	99	49	8.6	6.5
Perfection 1937.....	Daisy	10.2	15	9	25	10.1	.....	.....	54	30	4.5	3.3	57	21	5.2	5.4
Alaska 1937.....	Alaska	18.4	2	8	44	32.7	10.5	1.9	39	23	6.6	5.7	40	31	12.8	7.5
Alaska 1936.....	Alaska	18.0	0	7	41	41.0	4.5	10.5	42	31	5.6	4.5	35	25	10.1	6.7
Alaska 1935.....	Alaska	19.0	3	8	40	40.5	5.4	1.4	38	25	6.1	4.3	53	34	15.3	8.8
Stratagem 1938....	Alaska	9.8	17	4	23	34.7	29.0	10.0	50	28	6.4	4.7	.....	.....	.....	.....
Stratagem 1936....	Alaska	8.0	23	6	43	30.2	1.8	2.0	57	27	6.9	3.1	.....	.....	.....	.....
Perfection 1937.....	Alaska	10.2	15	10	67	30.0	10.2	2.3	50	22	5.2	2.1	33	18	7.9	5.7

* In 10^{-4} gamma/kg. indoleacetic-acid equivalents.

H. BORSOOK. The results in table 6 show that increase in total nitrogen is completely correlated with growth of the scion. Before the graft had taken, no change in nitrogen content of the scion occurred. But later, depending on how much growth had taken place, more or less nitrogen reached the scion. There was a corresponding decrease in the nitrogen content of the seed, but this decrease occurred also before the graft succeeded.

TABLE 6

NITROGEN FRACTIONS (AS DETERMINED BY DR. BORSOOK) OF SCIONS AND COTYLEDONS OF GRAFTED PEAS. TOTAL NITROGEN REFERS TO MG. OF NITROGEN PER TEN SCIONS OR TEN PAIRS OF COTYLEDONS

PERFECTION TIPS ON	TOTAL GROWTH (MM.)	GROWTH RATE BEFORE ANALYSIS (MM./DAY)			MAXIMAL GROWTH RATE (MM./DAY)	TIME BETWEEN GRAFTING AND ANALYSIS (DAYS)	NO. OF TIPS ANALYZED	SCIONS				COTYLEDONS OF STOCK			
								Total N	Percentage total N			Total N	Percentage total N		
		Three days	Two days	One day					Protein	Free amino	Amide		Protein	Free amino	Amide
(Before grafting)	.....	.....	.....	.....	.....	0	10	13.5	41	26	13	47.8	53	22	3
Perfection	4.3	0.2	1.0	2.8	3.0	11	10	13.4	22	30	16	38.6	42	28	6
Perfection	41.5	7.0	6.9	2.1	12.3	13	10	17.2	18	29	20	27.3	28	34	11
Perfection	46.5	8.7	7.8	9.3	12.0	13	10	25.7	20	32	19	21.9	24	37	11
Daisy....	1.2	0.6	0.0	0.3	0.8	10	10	12.9	17	31	21	23.2	34	32	9
Daisy....	45.4	6.4	6.4	7.0	12.5	18	5	23.2	19	29	20	.....	.....	.....	.....
Daisy....	85.4	7.2	7.2	8.0	16.4	18	5	36.9	19	31	20	.....	.....	.....	.....

This is comprehensible, since the roots continue to grow on such stocks. The last recorded value of nitrogen in cotyledons is not comparable with the others, since it refers to seeds of Daisy instead of Perfection. The protein, amino and amide fractions of the total nitrogen are rather constant in the scions, after an initial drop in protein, and do not vary with the root stock, although the stem and leaf growth rates are very different.

ANALYSIS OF GENETIC CHARACTERS BY GRAFTING TECHNIQUE

As mentioned before (12), it would be interesting to use as root stock peas with genetically-known aberrant leaf or growth characters. In this way it would be possible to determine whether a specific gene affects the growth-factor supply or the reactivity of the tissues.

The first such case was investigated by DE HAAN and GORTER (4). They found that the double recessive Slender character (la, lb according to DE HAAN, 3) owes its excessive length, at least partly, to a decreased auxin destruction, when compared with the dominant allelomorph. WENT (12) showed that in addition Slender seeds had a greatly reduced supply of leaf and stipule growth factors. A new experiment with the last available seed supply was carried out, with substantially the same results as previously reported (table 7). This table indicates that the non-Slender plants of the same family as Slender (Tall-and-Short), and probably the Slender plants as well, are a much better source for caulocaline than is Alaska. Whereas Tall-and-Short serves the scions with as much leaf and stipule growth factors as Alaska, the Slender stocks are deficient in these fac-

tors. The stem-growth response of Slender to caulocaline is the same as that of Alaska, but the reactivity of the stipule is definitely less. In Tall-and-Short the reactivity to caulocaline is decreased, showing that the Slender character is pleiotropic; it decreases auxin destruction, decreases leaf and stipule growth factors stored in seeds, and increases reactivity of the stem to caulocaline. Furthermore, according to DE HAAN (3) it decreases chlorophyll content, increases peduncle growth, and causes flower abnormalities.

Among other genetically-analyzed growth characters of the pea can be mentioned the Tall-Dwarf character, which expresses itself in the number and length of internodes, but Tall is not nearly so extreme as the Slender type of growth. This has not been investigated, neither has the Fasciated Stem.

A few cases of Stipuleless have been described. Two of them were investigated. One was Acacia-leaf, in which the leaves are multijugate by metamorphosis of tendril branches into leaflets. It also was Stipuleless. A number of these plants, received through the courtesy of Miss PELLEW and coming from different strains, were germinated and grafted with Alaska. Leaf growth was little affected by Stipuleless stock, and the stipule growth of Alaska scions was equal with both stocks. Stipuleless did not develop larger stipules on Alaska stock. This shows that Stipuleless is due to a lack of growth response of the primordium to stipule growth factors, which are supplied in sufficient amount by the cotyledons. In conjunction with decreased stem-growth rate, the petiole development was much smaller when Acacia-leaf was used as stock, and the same is true for tendril development. This may be attributable to the fact that in this case Stipuleless was of the Acacia-leaf type, which has small tendrils. The character of Acacia-leaf is not transmitted across a graft union, since the number of leaflets of Alaska was not increased by this root stock.

TABLE 7

STOCK AND SCION EFFECTS OF ALASKA PEAS GRAFTED ON OR WITH PEAS SHOWING A 3:1 SEGREGATION IN TALL-AND-SHORT AND SLENDER

STOCK	DATA	SCION		
		Alaska	Tall-and-Short	Slender
Alaska	Successful grafts	18	13	5
	Growth rate*	25.9±1.7	15.2±0.8	25.2±5.7
	Leaf length†	7.0±0.3	8.5±0.6	8.4±0.4
	Stipule length	7.3±0.2	7.6±0.2	5.8±0.5
	Petiole length	5.1	5.7	8.3
Tall-and-Short	Successful grafts	15	5	.....
	Growth rate	37.5±1.5	19.8±2.2	.....
	Leaf length	6.9±0.25	8.1±0.9	.....
	Stipule length	7.2±0.2	8.3±0.4	.....
	Petiole length	6.3	6.4	.....
Slender	Successful grafts	5	.....	.....
	Growth rate	36.0±4.2	.....	.....
	Leaf length	6.0±0.5	.....	.....
	Stipule length	6.2±0.4	.....	.....
	Petiole length	5.1	.....	.....

* Maximal rates reached by scions in mm./day. † Length measurements in mm.

In table 8 two experiments are summarized in which two genetic characters are compared in their effects on leaf and stipule growth. These races of Stipuleless were obtained through the courtesy of Dr. BARBER. There is a slight but significant decrease in stipule growth when Stipuleless stocks are used (table 8). Growth of leaves and stems, however, is not significantly influenced by this stock.

sponse of their different organs to growth factors as well as in the growth-factor content of their seeds. Both mechanisms contributed about equally to the size and shape of the seedling.

The case of Slender is complex, a situation in which reactivity of the leaves and stipules is decreased and that of the stem increased, together with a decrease of the growth-factor content of seeds and

TABLE 8

GROWTH RATES AND MEAN LEAF, STIPULE, AND PETIOLE SIZES OF FIRST TO THIRD LEAVES OF RECIPROCAL GRAFTS OF MUTANTS AND RACES OF PEA VARIETIES. MEAN OF TWO EXPERIMENTS

STOCK	SCION	NO. OF SUCCESSFUL GRAFTS	MAXIMAL GROWTH RATE		MEAN LENGTH OF FIRST TO THIRD NODES OF SCION			
			Mm./day	Reached after (days)	Leaf	Stipule	Petiole	Tendril
Alaska	Alaska	38	36	8.3	7.6	7.7±0.2	6.1	4.2
Duke of Albany (type)	Alaska	9	34	7.3	7.5	7.1	5.6	4.4
Duke of Albany Stipuleless	Alaska	32	35	7.3	6.9	6.0±0.2	5.1	3.2
Duke of Albany Rogue	Alaska	25	38	8.0	7.1	6.7±0.4	5.6	4.7
Alaska	Duke of Albany Stipuleless	21	39	7.2	7.7	2.3	6.8	6.3
Alaska	Duke of Albany Rogue	20	39	7.0	5.5	5.5	6.1	5.1
Duke of Albany Stipuleless	Duke of Albany Stipuleless	15	41	.....	7.0	2.4	8.0	.....
Duke of Albany Rogue	Duke of Albany Rogue	7	25	.....	5.2	6.1	6.0	.....

Rogue is a dominant character, which causes reduction of leaf and stipule growth. This character is due exclusively to reduced reactivity of the Rogue leaflets and stipules to leaf and stipule growth factors (table 8), and the latter are present in the cotyledons in the same amounts as in the parent variety from which the Rogue originated.

The results obtained in these tests with strains differing in only one gene are in marked contrast with a comparison between pea varieties as summarized in table 13 of the first paper of this series (12). In the latter case it was concluded that the varieties differed both in the response to an increase in the caulocaline production of the roots. The Slender habit is certainly more than the decreased auxin destruction described by DE HAAN and GORTER (4). It is evident that all these pleiotropic effects must be based on one much more fundamental effect of the Slender gene.

In the other three genes investigated (Stipuleless, Acacia-leaf, and Rogue) a simple relationship between gene and effect existed. In none of them was there a considerable change in the growth-factor content of the seeds. Practically the whole effect was due to a change in responsiveness of the tissues under control

of the gene. In *Zea mays* the growth substance analysis of a number of dwarf races (**8, 9**) gave a similar result; the dwarf genes did not affect the auxin content as such, or the auxin synthesis, but the dwarf habit was due mainly to excessive auxin destruction. Between various corn varieties, however, quantitative differences in auxin content exist.

These data indicate that differences in growth habit, which are due to known genes, are not primarily caused by differences in growth-factor synthesis or content. It is evident that growth-factor differences could account for major differences in habit, but also they would be controlled to a considerable extent by external conditions. There are two possible explanations of the one-sided nature of gene action in affecting the growth habit in peas:

1. Since growth-factor differences are so much influenced by growing conditions, clear-cut genetic data are hard to obtain, so that genes controlling the quantity of growth factors have not been picked up by geneticists.

2. Genes affecting size and form in peas are influencing the response of the individual cells to growth factors, or in corn they cause destruction of auxin. However, quantitative differences in growth-factor content do not seem to be controlled by known gene differences. It must therefore be assumed that the varietal differences in growth-factor content are due to more complicated genetic relations. In the few cases that quantitative characters have been genetically analyzed in plants, complex situations are encountered, calling for a whole array of multiple allelomorphs with modifying factors (**7**).

As soon as more genetic characters affecting size of stem, leaves, stipules, and tendrils of peas are known, this problem can be investigated in greater detail.

Growth-factor content of peas soaked in water

When peas are soaked in water for prolonged periods, leaf-growth factors leach out of the cotyledons (**2**). Such soaked peas germinate poorly or not at all. This compares with the behavior of peas sown in the field, which germinate very poorly if rain drenches the soil soon after sowing. The question was whether this poor germination could be accounted for by the depletion of growth factors in the cotyledons, or whether any tissues were injured so that no further growth could occur.

Superficially disinfected peas were submerged in an excess of water (about 100 seeds per 150 cc. of water) for various periods, partly without and partly with aeration. In the latter case a strong current of air ($\pm$ 100 cc. per minute) was bubbled aseptically through the container. After the period of soaking (1, 2, 3, 4, 6, or 8 days), the peas were laid out in the usual way on filter paper and after germination transferred to 20-cc. wide-mouthed bottles. When the plants had reached the proper length, all graft combinations between controls (soaked 4 hours only) and treated peas were made. In the first experiment, 201 out of 263 grafts succeeded; in the second, 190 grafts took. Since both experiments gave essentially the same results, only one will be described, based on the data of table 9. The upper three lines define the conditions to which the peas were subjected previous to germination. In groups 4 and 5 considerable growth of the seedlings occurred even while submerged, but the developing shoots were thinner and had very small leaves, stipules, petioles, and tendrils. When soaked for more than 50 hours in unaerated water, no further growth occurred after removal of the seeds from the solution (groups 8 and 9). From

line 4 it follows that considerable substance diffused from the peas, but part of this was reabsorbed by the developing seedlings in groups 4 and 5. Part of the soaked the more leaf-growth factor diffused out, especially when the water was not aerated. Lines 6–12 indicate the condition of the soaked peas after they

TABLE 9

GROWTH AND DEVELOPMENT OF PEAS SOAKED IN AERATED OR NONAERATED WATER, COMPARED WITH CONTROLS SOAKED 4-HOUR PERIOD ONLY (GROUP 1). UPPER 13 LINES REFER TO BEHAVIOR OF PEAS PRIOR TO GRAFTING. FROM 8–18 GRAFTS OF EACH COMBINATION WERE SUCCESSFUL. LOWER 10 LINES GIVE MEASUREMENTS OF SCIONS OF SUCCESSFUL GRAFTS. LINES 14, 17, 19, AND 22 REFER TO GRAFTS IN WHICH STOCK BELONGED TO SOAKED GROUPS AND SCION TO CONTROLS, WHEREAS IN LINES 15, 18, 21, AND 23 THE STOCKS WERE CONTROLS (FROM GROUP 1) AND THE SCIONS SOAKED

LINE	DATA			GROUP								
				1	2	3	4	5	6	7	8	9
1	Hours soaked			4	24	48	96	144	24	48	96	144
2	Aerated or not			.....	Air	Air	Air	Air	No air	No air	No air	No air
3	Subsequent germination			++	++	++	+	+	+	+	−	−
4	Diffusate in soaking water (mg.)			.....	45	62	48	30	65	83	98	240
5	Amount of leaf-growth factor in diffusate (percentage growth)			0	1.3	1.3	2.3	2.3	2.7	3.3	4.3	5.7
6	Hours after beginning of soaking for seedlings to reach 30 mm. length			131	137	159	189	223	168	205	.....	.....
7	Lag of treated seedlings behind controls (hours)			.....	6	28	58	92	37	74	.....	.....
8	Growth rate of seedlings (20–50 mm.)			36	30	36	36	.....	33	40	.....	.....
9	Development of organs at fourth node	Length in mm.	Leaf	8.1	8.1	6.7	5.4	4.5	6.8	6.4	.....	.....
10			Stipule	7.3	6.9	5.4	4.4	3.8	5.7	5.2	.....	.....
11			Petiole	7.8	7.2	4.6	3.4	2.8	4.3	4.2	.....	.....
12			Tendril	4.7	3.0	2.6	1.5	1.3	2.7	2.1	.....	.....
13	Diameter of stem of soaked seedling			2.28	2.28	2.24	2.04	2.16	2.20	2.18	.....	.....
14	Effects of stock on scion development	Maximum growth rate (mm./day) when soaked seedling is	Stock	40	39	35	40	40	37	39	.....	.....
15			Scion	40	40	39	40	40	36	42	.....	.....
16			Both stock and scion	40	38	38	38	.....	39	.....	.....	.....
17		Leaf (mean of third and fourth leaves) length (mm.) soaked seedling being	Stock	7.0	7.0	7.1	6.5	6.7	6.3	5.6	.....	.....
18			Scion	7.0	7.3	7.0	6.3	6.4	6.9	7.1	.....	.....
19			Both stock and scion	7.0	.....	7.3	6.2	.....	6.0	.....	.....	.....
20		Mean length of third and fourth stipules when soaked seedling is	Stock	7.1	7.1	7.7	7.3	6.9	7.4	6.5	.....	.....
21			Scion	7.1	7.0	7.3	7.3	7.1	6.9	7.4	.....	.....
22		Mean length of third and fourth petioles when soaked seedling is	Stock	10.7	9.0	7.9	7.3	6.4	7.8	4.8	.....	.....
23			Scion	10.7	8.3	7.3	6.1	6.7	8.7	8.3	.....	.....

material diffusing out was leaf-growth factor, as the determinations of line 5 show (carried out by Dr. D. BONNER with the standard leaf-growth test method, 1). The longer the peas were had germinated and grown. Soaking greatly retarded early growth of the seedlings, so that it took 6–92 hours longer than the controls to reach a length of 30 mm. But once they started to grow

their growth rate was normal and approximately the same, irrespective of soaking. Only those seedlings which developed normal roots started to grow, whereas those with roots permanently injured by the soaking hardly grew at all. This supports the view earlier expressed (13) that only in actively growing pea roots is an essential growth factor for stems (caulocaline) formed. As can be seen from table 9, there were great differences in the appearance of the seedlings, even though they grew at the same rate: the longer they were soaked the less leaf, stipule, petiole, and tendril development occurred, and the thinner were the stems.

After grafting, the growth rates of the scions were the same, whether soaked peas were used as stock, as scion, or as both stock and scion (table 9). The remarkable uniformity of the maximal growth rates indicates how well the grafting in this experiment succeeded (201 out of 263 grafts took—a mean of 12 per group). To judge the effect of the grafting on leaf, stipule, and petiole development, the mean length of the third and fourth leaves on each graft was taken. From the third leaf on, the effect of the soaking itself has mostly worn off, so that the stock effects become apparent. It will be seen that especially the non-aerated peas when used as stock decrease leaf and petiole growth of the nonsoaked scion. If the leaf length of the fourth leaves of nonsoaked scions grafted on groups 4, 5, 6, and 7 (6.66 ± 0.20) is compared with the length of the fourth leaves of scions grafted on controls (group 1: 7.50 ± 0.20), then the difference between these groups is highly significant ($\Delta = 0.84 \pm 0.27$), indicating that the content of leaf-growth factor has significantly decreased during soaking, which was to be expected since these factors could be collected from the water in which the material was soaked. Comparison of lines 5 and 17 shows that there is a rough proportionality between the amount of leaf-growth factors in the water and the amount which disappeared from the cotyledons. The decreased leaf development in groups 4 and 5, line 18, was due mainly to incomplete recovery of the third leaf from soaking, since the fourth leaves were already of almost the same size as those in the control grafts.

Conclusions reached from this experiment, confirmed by those of another, are that soaking peas for prolonged periods decreases subsequent germination, especially when the water is not aerated. The effects of this soaking are either (*a*) transitory or (*b*) permanent. Whenever the roots have been injured beyond recovery, no further stem growth is possible. But when the roots recover, growth of the stem, both prior to and after grafting, becomes normal and maximal. The inhibition of growth in length during the soaking period is therefore transitory and can be traced back to temporary injury to the roots resulting in a temporary decrease in caulocaline production. The decreased development of leaves, stipules, petioles, and tendrils in the soaked material is not due to injury to the cells, for when grafted on the control seedlings their growth becomes normal again. Consequently, the smaller size of these organs has to be attributed to a decreased supply of growth factors, which leach out into the water; the leaching is much greater for the shoots than for the cotyledons.

This soaking produces the same condition of reduced organ size in peas as some of the genetic characters which decrease leaf and stipule growth. But whereas the growth genes cause a de-

creased reactivity of the cells, without affecting the leaf-growth factors, soaking mainly decreases growth factors without affecting the reactivity of the cells.

Summary

1. The percentage of successful grafts of pea seedlings grown in darkness at 24° C. and 80 per cent humidity drops rapidly when the plants are more than 8 days old, both when used as stock or as scion. No considerable improvement in percentage of take or of subsequent maximal growth rate is observed when the cut surfaces of the graft partners are treated with plant hormones. When stem pieces of varieties which as stock produce considerable differences in scion growth are grafted as intermediate stems between pieces of one test variety, they do not influence the scion. The effect of stock on scion is exclusively due to cotyledons and root system and not to transport properties of the stem or to the graft union, once this is established.

2. There is no correlation between auxin content of the tip on the one hand and stem growth rate and stock variety on the other hand, indicating that the growth rate in these grafts is not limited by auxin but by another factor coming from the stock. This is caulocaline, as concluded in the first paper of this series.

3. The decrease in viability of pea seeds upon storage is not due to a gradual decrease in growth factors present in the cotyledons. The only observable effect of age is decreased resistance to premature rotting of the seeds.

4. The protein content of the scion follows very closely its growth rate.

5. The genetic characters Stipuleless, Acacia-leaf, and Rogue are not accompanied by appreciable changes in growth-factor content of the stocks; they are due almost entirely to changes in reactivity of the tissues. On the other hand, decreased development of leaves, stipules, petioles, and tendrils due to soaking of the peas in water for 1–6 days is caused by decrease in growth-factor content of the cotyledons and not by changes in reactivity of the tissues to growth substances.

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EFFECTS OF APPLICATION OF THIAMINE TO COSMOS[1]

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Introduction

Earlier experiments (**2, 3**) were thought to indicate that the application of thiamine to selected species of plants might result in increased growth under the environmental conditions used. In general, other investigators have not found such effect (**4, 1, 5**), although occasionally positive responses have been recorded (**6, 7**). The present investigation has been a study of some of the factors influencing the growth of *Cosmos sulphureus*, and the relation of these factors, if any, to the apparent growth response of *Cosmos* to added thiamine. In all, eighty-five experiments with the application of thiamine were conducted. In fifty of these experiments, growth responses which appeared to be significant were obtained. In the remaining thirty-five experiments no significant responses appeared. Of the several factors whose influence on the growth of *Cosmos* was investigated, certain ones appeared to bear little relation to any apparent response to thiamine. This was true of the pH of the nutrient medium over a considerable range, varying degrees of nitrogen deficiency, and light intensity within wide limits. The temperature relations of the conditions under which *Cosmos* was maintained, however, appeared to be of great importance in the growth of this plant.

Material and methods

All plants grown in sand culture were contained in 2-gallon glazed crocks provided with drainage. In most of the experiments a local washed river sand, having particles between 10 and 20 mesh, was used. Growth in this medium appeared to be as luxuriant as in 4-mesh gravel or in 4-mesh quartz gravel. Twenty seeds of *Cosmos*, var. Klondyke orange flare, were planted per crock. When the first foliage leaves were well developed (in general, 7–10 days after planting), the experiment proper was commenced. Thirty days later the plants were harvested, the tops separately from the roots, dried rapidly in a current of dry air at 60° C., and weighed.

Hoagland's nutrient solution containing the micro-nutrients was applied at the rate of about 500 cc. per crock per day or per 2 days. Experiments with varying frequency of nutrient application were conducted periodically throughout the investigation, and in no experiment—with the possible exception of C_{75} (table 4)—was growth limited by the amount of available nutrient. Additional water was supplied as needed, and the crocks were periodically flushed with water.

No consistent difference between growth of plants supplied with distilled water and that of plants supplied with tap water was found, but in most of the experiments distilled water was used. Thiamine was applied in the nutrient solution at the rate of 0.01 mg. per liter.

Two greenhouses were employed. In the first the temperature was controlled only in so far as a night minimum of 55° F. was maintained. In the second, an air-conditioned greenhouse, constant temperatures and humidities could be maintained. The experiments in the air-conditioned greenhouse were carried out at a relative humidity of 70 per cent, regardless of temperature.

[1] Report of work done with the assistance of the Work Projects Administration, O.P. no. 165-1-07-172. This work was made possible in part by the support of Merck and Company.

A randomized block design was employed with all experiments. At the time of harvesting, the ten plants of each crock were divided into two lots of five plants each. Treatment of the data from typical experiments by the analysis of variance showed that variation between the two lots from the same crock was not significantly different from that between two lots from different crocks. The unit of five plants is therefore used as the basis for the computation of the standard errors in the tables. Any comparison, unless otherwise stated, is attended by fourteen or more degrees of freedom. The word significant is used only in its statistical sense.

TABLE 1

DEVELOPMENT OF COSMOS PLANTS WITH AND WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

EXPERIMENT NO.	TOTAL NO. OF PLANTS	DRY WEIGHT OF TOPS (GM. PER FIVE PLANTS)			DRY WEIGHT OF ROOTS (MG. PER FIVE PLANTS)		
		Control	Thiamine	Difference	Control	Thiamine	Difference
C48....	160	0.350±0.0138	0.485±0.0295	0.135±0.0326*	35.0±2.60	38.8±2.92	3.8±3.90
C49....	160	0.505±0.0214	0.655±0.0294	0.150±0.0364*	47.2±4.4	60.0±5.95	12.8±7.40
C50....	160	0.419±0.014	0.592±0.039	0.173±0.0413*	45.0±4.0	72.5±6.35	27.5±7.50*

* Difference significant at 1 % level.

Experimental results

REPETITION OF EARLIER EXPERIMENTS.—In certain experiments an attempt was made to repeat as closely as possible those reported earlier (3). *Cosmos* plants were grown in sand, ten plants per crock, in 2-gallon crocks in the greenhouse, and harvested after 30 days. Table 1 gives the data from three such experiments. Significant increases in dry weight of tops appear to have been obtained as a result of treatment, amounting to 39, 30, and 41 per cent, respectively, in the three experiments. Effect of the treatment on dry weight of roots was variable and not significant in two of the three experiments. This experiment was repeated fifteen times and yielded a mean increase of dry weight of tops as a result of treatment of 27 ± 4.4 per cent, a highly significant result. Although the effect of the treatment varied from experiment to experiment, it would appear that under the conditions used a small but significant increase in dry weight of tops resulted from thiamine application in individual experiments and in the whole series.

EFFECT OF TEMPERATURE.—It has been pointed out by HAMNER (4) that the *Cosmos* plants grown in the earlier experiments of BONNER and GREENE (3) exhibited less luxuriant growth than those grown by himself. The same criticism applies to the plants of table 1. This low rate of growth was not due to a limitation of the available nutrient, but was related to the relatively low temperatures which were, for other reasons, maintained in the greenhouse. Thus, the nightly minimum through the winter months, during the earlier experiments, was approximately 55° and the daily maximum approximately 75° F. In preliminary experiments it was found that when *Cosmos* plants, under otherwise similar conditions, were allowed to develop either at the aforementioned temperature conditions or at a constant temperature (80° F.), growth was consider-

ably greater at the higher temperature. Experiments at varying temperatures were therefore undertaken. Two units of the air-conditioned greenhouse were set for constant temperatures of 80° and 68° F., respectively. In addition, plants were transferred between the two units, so that an 8-hour period per day at one temperature was combined with a 16-hour period per day at the other temperature. In all, ten experiments, each including treatment with two or more different temperature combinations, were carried out during two successive winters. Data from two such experiments are summarized in table 2. Plants maintained continuously at 68° appeared to show a small response to thiamine treatment. Plants maintained at 80° or which received a temperature of 80° during the night and 68° during the day showed no significant response to the treatment with thiamine.

In the two experiments of table 2, growth does not appear to be greatly affected by the several temperature treatments. When a day temperature of 80° was combined with a night temperature of 68°, however, growth was measurably improved over that obtained at a continuous temperature of 68° (table 3). In the four experiments of table 3, growth at a continuous temperature of 68° was greater than that in the comparable experiments of table 2, owing to factors as yet unknown. With a day temperature of 80° and a night temperature of 68°, growth was still greater. As in the experiments of table 2, a significant or highly significant response to thiamine treatment (with respect to dry weight of tops produced) was obtained at the continuous temperature of 68°. Plants which received a day temperature of 80° and a night temperature of 68° exhibited no response to the treatment.

TABLE 2

DEVELOPMENT OF COSMOS PLANTS AT 80° AND 68° F. WITH AND WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

Experiment no.	Temperature		Total no. of plants	Dry weight of tops (gm. per five plants)			Dry weight of roots (gm. per five plants)		
	Day	Night		Control	B_1	Difference	Control	B_1	Difference
C60	68°	68°	80	0.95±0.065	1.30±0.047	0.35±0.080*	0.22±0.018	0.34±0.012	0.12 ±0.022*
C66	68°	68°	80	0.80±0.063	1.18±0.080	0.38±0.102*	0.16±0.022	0.20±0.019	0.040±0.029
C60	68°	80°	100	0.91±0.064	1.04±0.042	0.13±0.077	0.24±0.020	0.28±0.017	0.040±0.026
C66	80°	80°	80	1.08±0.12	1.18±0.15	0.10±0.19	0.15±0.018	0.16±0.023	0.010±0.029

* Difference significant at 1% level.

TABLE 3

DEVELOPMENT OF COSMOS PLANTS AT 80° AND 68° F. WITH AND WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

Experiment no.	Temperature		Total no. of plants	Dry weight of tops (gm. per five plants)			Dry weight of roots (gm. per five plants)		
	Day	Night		Control	B_1	Difference	Control	B_1	Difference
C67	68°	68°	80	2.30±0.12	3.21±0.14	0.91±0.18*	0.33±0.054	0.46±0.038	0.13 ±0.066
C71	68°	68°	40	2.65±0.13	3.18±0.15	0.53±0.20†	0.39±0.069	0.35±0.076	0.040±0.103
C81	68°	68°	300	2.24±0.070	2.70±0.087	0.46±0.11*	0.28±0.011	0.34±0.017	0.06 ±0.020*
C84	68°	68°	120	2.72±0.11	3.55±0.12	0.83±0.16*	0.39±0.022	0.40±0.020	0.010±0.030
C67	80°	68°	80	4.95±0.24	4.70±0.20	0.25±0.31	0.94±0.13	1.04±0.12	0.10±0.18
C81	80°	68°	120	5.73±0.30	6.30±0.29	0.57±0.42	0.76±0.065	0.74±0.051	0.02±0.082

* Difference significant at 1% level. † Difference significant at 5% level.

Dry weight of roots was affected variously, a significant result being obtained in only one case.

These experiments indicate that under the prevailing conditions the temperature relations may affect not only the growth of *Cosmos* but also its apparent response to thiamine treatment. Those temperature relations favoring more rapid growth do not appear to be conducive to any apparent response to thiamine (table 3).

LUXURIANT GROWTH.—Some effort was made to discover conditions favoring a growth of *Cosmos* still more luxuriant than that recorded in table 3. The number of plants per 2-gallon crock was reduced from ten to four, although this alteration resulted in but slight increase in growth. The most luxuriant growth as yet obtained was in midsummer and with the use of the system described by WENT (8), in which the prevailing temperature is adjusted to the prevailing light intensity. The maximum temperature in the greenhouse under full sunlight was approximately 90°, while the nightly minimum was 65°. The results of this experiment are given in table 4 (experiment C75). At the expiration of 30 days the plants were more than 3 feet tall, about $\frac{1}{2}$ inch in diameter, and the dry weight of the tops was several times greater than that of the largest plants of table 3. No apparent response to thiamine was evident. Repetition of the experiment failed to duplicate such growth, and in one case (table 4, experiment C79)—carried out in the fall—a significant response to thiamine was apparent. Relatively rapid growth was also obtained in unaerated solution culture (table 4), in which case, in confirmation of the findings of ARNON (1), no response to thiamine was apparent.

TABLE 4

DEVELOPMENT OF COSMOS PLANTS UNDER VARIOUS CONDITIONS AND WITH OR WITHOUT ADDITION OF THIAMINE TO NUTRIENT SOLUTION

EXPERIMENT NO.	ENVIRONMENT	TOTAL NO. OF PLANTS	DRY WEIGHT OF TOPS (GM. PER FIVE PLANTS)			DRY WEIGHT OF ROOTS (GM. PER FIVE PLANTS)		
			Control	B_1	Difference	Control	B_1	Difference
C75.....	Balanced light temperature system (July)	120	21.1 ±0.78	21.8 ±0.75	0.7 ±1.08	3.38±0.20	3.50±0.22	0.12±0.30
C79.....	Balanced light temperature system (September)	96	7.10±0.26	8.75 ±0.35	1.65±0.43*	1.12±0.10	1.40±0.16	0.28±0.19
C78.....	Solution culture	36	7.88±0.415	7.72±0.285	0.16±0.503	2.21±0.157	2.14±0.169	0.07±0.231

* Difference significant at 1% level.

Discussion

Significant increases in growth of tops of *Cosmos* plants were obtained in certain experiments apparently as a result of the application of thiamine. Response to added thiamine, however, depended on the conditions under which the plants were grown. Of the environmental factors affecting the response, temperature would seem to be of greatest importance, so far as the present experiments are concerned. On the whole, temperature relations conducive to luxuriant growth would appear to be unfavorable for demonstration of any apparent response to added thiamine.

Even in experiments where apparent growth responses to added thiamine were found, these responses were smaller

than those brought about by suitable changes in temperature. Thus, at a continuous temperature of 68° F. the addition of thiamine in six experiments resulted in an apparent increase of 33 per cent in dry weight of tops, whereas subjecting similar plants to 8 hours per day at 80° resulted in an increase of more than 100 per cent.

The present discussion has centered on the growth responses of the tops of the plants, since responses in dry weight of roots were variable and in general insignificant (tables 1–4). No explanation of this fact can be given at present.

It is recognized that these experiments should be expanded to cover further temperatures and temperature combinations, as well as further variation of other environmental factors in combination with temperature. It is also recognized that other factors, as yet unknown, may exert important influences in determining the outcome of such experiments. It is possible, however, that the data presented here may suggest a partial explanation of the discrepant results obtained from thiamine applications to plants by other investigators.

The results of the present study are not held to bear on the question of the lack of practical value of promiscuous thiamine application to plants. Considerable published and unpublished evidence has established that such applications are without general merit.

Summary

1. Experiments on the application of thiamine to *Cosmos* plants were repeated under conditions similar to those obtaining in previous experiments (3). Addition of thiamine to the nutrient solution appeared to elicit small growth responses, as judged from dry weight of tops produced.

2. The relatively slow growth rate obtained in the earlier experiments (3) was found to be owing to the relatively low temperatures under which the plants were grown.

3. Thiamine was applied to plants grown at controlled temperatures of 80° and 68° F. Positive growth responses to added thiamine appeared to be obtained at a constant temperature of 68° but not of 80° F., nor at a day temperature of 80° combined with a night temperature of 68° F.

4. Temperature conditions favoring luxuriant growth appeared unfavorable for demonstration of any apparent growth-promoting effect of thiamine.

5. Although temperature conditions, in so far as they were studied, appeared to affect the apparent response to thiamine, it is recognized that other factors may have similar and possibly more striking effects.

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RELATIONSHIP OF DISSOCIATION OF CELLULAR PROTEINS BY INCIPIENT DROUGHT TO PHYSIOLOGICAL PROCESSES

HENRY T. NORTHEN[1]

(WITH ONE FIGURE)

Introduction

When cellular proteins are subjected to environmental changes, the native proteins may be changed into labile ones which may be dissociated, associated, or denatured (27). The data presented here indicate that incipient drought, an experience common to plants, conditions a dissociation of protoplasmic proteins. This generally results in decreased resistance to lethal agents, increased protoplasmic swelling pressure, accelerated rates of respiration and polysaccharide hydrolysis, and may at times lead to mitotic abnormalities (27).

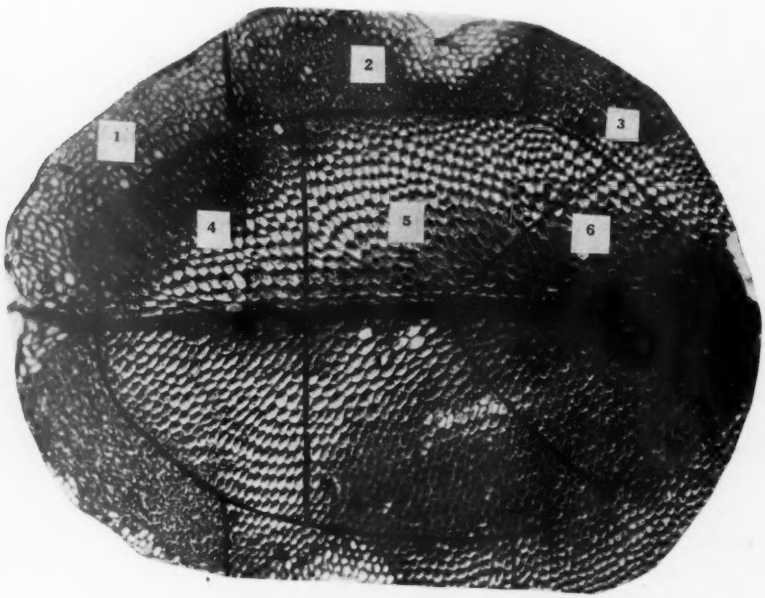

FIG. 1.—Fourth leaf, dried 20 minutes. Controls exhibited no displacement

MATERIAL AND METHODS.—Gametophytes of mosses, *Mnium* sp. and *Bryum* sp., were placed in a desiccator over anhydrous calcium sulphate. After exposures of 10–50 minutes, the plants were removed and centrifuged with an acceleration of 680× gravity for 3 minutes, a centrifugation which never displaced the chloroplasts in leaf cells of control plants. Next the approximate

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 192.

percentages of cells in which the chloroplasts were displaced were recorded for various zones (fig. 1) of the first sixteen leaves, the leaf nearest the stem tip being designated 1, the next 2, and so on. In other experiments with *Mnium* the gametophytes were dried for 2 hours, by which time they were completely shriveled. They were then transferred to water and centrifuged after various periods. Data were obtained as previously described.

TABLE 1

EFFECTS OF DROUGHT ON STRUCTURAL VISCOSITY OF PROTOPLASM

Leaf number	Minutes in desiccator	Percentage of displacement in zone					
		1	2	3	4	5	6
4	*						
	10	100	0	0	75	0	0
	20	0	0	100	100	100	40
	30	0	0	0	0	75	100
	40	0	0	0	0	0	0
	50	0	0	0	0	0	0
8	10	0	0	0	0	0	0
	20	100	100	25	100	100	100
	30	0	0	100	0	25	100
	40	0	0	0	0	0	0
	50	0	0	0	0	0	0
12	10	0	0	0	0	0	0
	20	0	50	75	25	50	100
	30	75	100	75	100	100	100
	40	0	50	100	100	100	100
	50	0	0	0	0	0	0

* Controls: 0% in all zones of all leaves.

Data

The data for only the fourth, eighth, and twelfth leaves for one of eight according experiments with *Mnium* are recorded in table 1. As water is evaporated, the percentages of cells in which the chloroplasts were displaced become greater, but with further loss the percentages decrease. Hence during the initial stages of drought the structural viscosity of the protoplasm decreases, whereas in more advanced stages it increases. Whether the protoplasmic viscosity becomes greater than normal could not be determined, because the highest centrifugal acceleration available, 1062× gravity, would not displace the chloroplasts in control cells. However, FRITSCH and HAINES (8) and NORTHEN (25) previously noted that prolonged drought conditioned a higher-than-normal protoplasmic viscosity. It is probable that decreases in viscosity are conditioned in part by dissociations of protein molecules (splitting into molecules of smaller size), and that increases result from subsequent associations—that is, aggregation into molecules of greater size (27).

TABLE 2

EFFECTS OF PREVIOUS DROUGHT ON STRUCTURAL VISCOSITY OF PROTOPLASM

Leaf number	Minutes in water after drying	Percentage of displacement in zone					
		1	2	3	4	5	6
4	*						
	15	0	0	0	0	0	50
	30	0	0	0	0	100	100
	60	0	0	0	0	0	0
	90	0	0	0	0	0	0
8	15	0	0	0	50	100	100
	30	0	0	0	100	100	0
	60	0	0	0	0	0	0
	90	0	0	0	0	0	0
12	15	0	0	75	100	100	100
	30	0	0	0	100	100	100
	60	0	0	0	0	0	0
	90	0	0	0	0	0	0

* Controls: (1) leaves immersed in water but not previously dried; 0% displacement in all zones of all leaves; (2) leaves after 2 hours of drying; 0% displacement in all zones of all leaves.

Abridged data for recovery experiments with *Mnium* are recorded in table 2. As evidenced by greater percentages of displacement than in controls, the pro-

teins become dissociated during the recovery period.

When gametophytes of *Bryum* sp. were subjected to drought, the results (a decreased protoplasmic viscosity followed by an increase to at least normal) were similar to those obtained with *Mnium*.

Discussion

Because the viscosity of many hydrophilic and hydrophobic sols *in vitro* does not decrease but rather increases as the concentration is augmented (20), the data support the previously advanced suggestion (27) that decreases in protoplasmic viscosity are conditioned in part by dissociations (disaggregations) of protein molecules. The fact that some protein molecules *in vitro* may be dissociated by increasing the concentration also supports this view. For example, PEDERSEN (30) noted that protein molecules could be split into molecules one-eighth of the original size by increasing the concentration.

Agents which dissociate protoplasmic proteins often condition an increase in the rate of respiration (27). When dissociation occurs a number of linkages are broken, salt linkages, hydrogen bonds, and of particular importance, disulphide linkages. When the latter are broken, sulfhydryl groups, which are activators of several respiratory enzymes (9, 12, 32, 33), are formed. Because with few exceptions enzymes are proteins or breakdown products of proteins (40), it is possible that they will be disunited from their combination with other proteins when dissociation occurs. Furthermore, respiratory substrates may become disengaged as a result of dissociation. If it be true that agents which condition dissociation make circumstances more favorable for respiration through an increase in such respiratory activators as -SH groups and through the disengagement of respiratory enzymes and substrate molecules, incipient drought should condition an increase in the rate of respiration. During the initial stages of drought, respiration is often increased in plants (19, 24, 29, 36, 37) and at times in animals (4, 7, 17, 18, 35). The data of PALLADIN and SHELOUMOVA (29) show all the expected consequences. They noted that the intensity of respiration of drying potato tubers first increased but with further drying decreased. Apparently the increase was conditioned by dissociation and the decrease by association. Furthermore, dried tubers, which had a decreased rate, respired more rapidly than controls when they were supplied with moisture. It will be recalled that the proteins became dissociated when dried moss leaves were again supplied with moisture.

The structural alterations of protoplasm which condition an increased respiratory rate may also condition an increased rate of polysaccharide hydrolysis, and indeed at times this may be partly responsible for the accelerated respiration (27). It is significant that carbohydrases, like respiratory enzymes, are actuated by -SH groups (15, 21). In addition, dissociation may result in the disengagement of carbohydrases from other proteins. KURSSANOV (16) states that enzymes are partitioned in the cell between the structural elements and the aqueous solution. In solution the enzymes hydrolyze and, when adsorbed, synthesize. Furthermore, he believes that changes in the physico-chemical condition in the cell may modify the ratio. When protoplasmic proteins dissociate, carbohydrases may accumulate in the aqueous phase, thus conditioning hy-

drolysis. If it be true that the dissociation of cellular proteins makes conditions favorable for polysaccharide hydrolysis, incipient drought should condition an accelerated rate. Polysaccharide hydrolysis is accelerated by wilting (1, 10, 13, 23) and by plasmolysis (14), which, like incipient drought, conditions a dissociation of cellular proteins (26).

The dissociation of cellular proteins by drought or hypertonic solutions augments the swelling pressure of protoplasm (27). This may account in part for the anomalous osmotic behavior of various cells in hypertonic solutions, although in such instances increased permeability and the production of osmotically active substances are other factors. With *in vitro* protein systems, the dissociation of protein molecules, conditioned by an increase in concentration, results in an augmented osmotic pressure (30). With respect to animal cells, BELKIN and SHEAR (2) noted that viable spindle cells swelled more in hypertonic solutions than they did in hypotonic ones. SOLLMANN (38) reported that the intravenous injection of a hypertonic solution produced at first an increase in brain volume. COOKE (5), ORT and MARKOWITZ (28), and HEILBRUNN (11) noted that muscle tissues in hypertonic solutions behaved as though they had a higher osmotic pressure than the solutions for which they were isotonic. In hypertonic solutions erythrocytes often behave anomalously (6, 31, 34). ROEPKE and BALDES (34) suggested that such irregularity may be partly the result of a dissociation of hemoglobin molecules, an explanation in harmony with the concepts developed here. For plant cells, WELCH (42) noted that the margins and bases of leaves of *Bryophyllum* which had dried for 11 weeks contained greater percentages of water and hence were more turgid than leaves from well-watered plants. BUHMANN (3) observed, contrary to expectations, that 37.8 per cent of the epidermal cells of *Bergenia cordifolia* increased in size in hypertonic sucrose solutions. Apparently in such cells the proteins were dissociated, and as a result the protoplasm imbibed water, a consequence of the increased protoplasmic swelling pressure.

Mitotic abnormalities may result from osmotic dehydration (39, 41, 44) or incipient drought (22, 44). MILOVIDOV (22) noted that the evaporation of water from root tips conditioned a dissolution of the phragmoplasts, and as a consequence cells with more than one nucleus were produced. Subsequent nuclear fusions resulted in the production of polyploid nuclei. The dissolution of the phragmoplasts probably resulted from dissociation and subsequent swelling. With respect to the formation of polyploid nuclei, it is significant that the widely used colchicine, like incipient drought, conditions a dissociation of cellular proteins—as evidenced by decreases in protoplasmic viscosity (43).

Summary

1. The centrifuge method was used to determine the structural viscosity of protoplasm in leaf cells of *Mnium* sp. and *Bryum* sp. which had been dried for periods up to 50 minutes. In other experiments with *Mnium*, dried plants were placed in water and the viscosity determined after periods varying from 15 to 90 minutes.

2. Incipient drought conditioned a decrease in the structural viscosity as a consequence of protein dissociations. During the recovery period a decrease in viscosity preceded the return to normal.

3. It is suggested that the dissociation of cellular proteins by incipient drought conditions an increased protoplasmic swelling pressure, accelerated rates of respiration and polysaccharide hydrolysis, and may at times lead to mitotic abnormalities.

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DEFOLIATION OF TOMATO PLANT AS A RESPONSE TO GASEOUS EMANATIONS FROM THE FRUIT[1]

JOHN SKOK[2]

Introduction

Defoliation of tomato plants during the latter part of the growing season is a major problem in eastern and midwestern tomato-producing areas. This response usually becomes evident when fruits start ripening and progresses rapidly as larger amounts of fruit are produced. It has been ascribed to a number of causes, the more important being leaf-spot diseases, unbalanced nutrition, and abnormal physiology (1, 5, 6, 7). The leaf-spot diseases have generally been regarded as the main cause, although spraying with fungicides seldom entirely prevents defoliation, and in many cases the effect is slight (5, 6).

ANDRUS *et al.* (1) and HORSFALL and HEUBERGER (5) have pointed out that fruit-load is concerned with susceptibility to defoliation, and SAMSON (8) states that defoliation coincides with the period of "heavy draft of the fruit on plant food resources."

A number of preliminary experiments and observations suggested that defoliation is in part a response to emanations of ethylene or of a combination of ethylene and other unsaturated hydrocarbon gases from ripe fruits underneath the vines. Minute traces of ethylene are present in the gaseous emanations from various plant tissues (3, 4), especially ripening fruits. The tomato plant is extremely sensitive to ethylene (2).

[1] These investigations are being conducted under a grant from Libby, McNeill, and Libby to the Department of Horticulture of the University of Illinois.

[2] Associate in Vegetable Crops, Department of Horticulture, University of Illinois.

Observations

Defoliation usually starts with shedding of those leaves nearest the crown of the plant in close proximity to the first ripened fruits. Progressive defoliation proceeds toward the terminal ends of the branches as subsequent clusters of fruits ripen. After defoliation has become extreme, only the leaves at the tips of the branches remain, that is, those which are beyond the clusters of ripe fruits.

Tomato fields almost bare of green leaves after severe defoliation often become green again in the latter part of the growing season, owing to new shoot growth in the axils of the leaf scars on the old vines. By that time most of the fruits have decayed or dried up, and apparently new growth is possible as soon as they are eliminated, weather conditions permitting. Those vines which fail to produce new shoots still have ripe fruits on them or in close proximity. The supply of nutrients and moisture is ample for growth.

During the last summer it was observed that plants grown in a number of home gardens generally failed to defoliate to the same extent as those in large commercial fields. From such garden plants the fruits were removed promptly upon ripening, whereas the fruits in the large fields were not always picked at the proper time; large quantities of over-ripe fruits accumulated, and the plants became defoliated.

Experimentation

These various observations suggested a possible relationship between fruit ripen-

ing, gaseous emanations from such ripe fruits, and defoliation. Experiments were devised which would test the hypothesis.

EXPERIMENT 1.—Young potted tomato plants placed in glass-inclosed chambers together with a number of ripe tomato fruits showed epinasty which became very severe after 18 hours. Induced epinasty by emanations from ripe fruits is probably mainly due to the presence of ethylene (2, 3). Two days after the plants had been placed in the chambers a number of the lower leaves abscissed, and on the fourth day only the top leaves remained, and these showed pronounced epinasty. On the sixth day most of the treated plants were dead. Control plants in similar chambers containing no ripe fruits showed none of these changes.

EXPERIMENT 2.—Late in August a number of young potted tomato plants were transplanted at random in a field of Early Baltimore tomatoes which were in full bearing and showed marked defoliation. The young plants were set near the old ones and so arranged that a number of ripe fruits were directly underneath them. A control group of young plants of the same lot was transplanted in the open at a distance of about 20 feet from the edge of the field and not near any ripening fruit.

After 4 days the young plants set close to the bearing plants had become pale green and some of their leaves and leaflets were curled, although there was no pronounced epinastic response. On the fifth day after transplanting the lower leaves were distinctly yellowed and curled, and the tips of several leaflets had dried. The following day or the day after, all the lower leaves died and dried up; the upper leaves were curled and had developed dried tips and edges. Some of the older leaves had abscissed, but in most cases the blades were dry and the petioles remained attached for a time and abscissed later. This curling, drying, and abscission is very similar in appearance to the usual defoliation which takes place in older fruit-bearing plants.

About 15–16 days after transplanting, the remaining upper leaves of the young plants in the field, which had been pale, curled, and dried along the tips and edges, seemed to regain color and resume growth, and the plants grew considerably in height in the next day or two. The fruits underneath these plants had dried by this time. Additional fresh fruits were placed there, but no apparent response was noticeable. The new leaves of the plants were 12–18 inches above the fruits at this time.

In addition to curling, drying, and abscission of leaves, spotting of the leaves also developed. These spots were present on the upper curled leaves as well as on the lower dried ones but were entirely absent from any leaves of the control plants. Microscopic examination of these areas showed the presence of spores of both *Alternaria* and *Septoria*. The presence of fruit emanations may have some effect in increasing the susceptibility of the tomato plant to these pathogens.

During the entire period the young control plants adjacent to the field remained green, vigorous, and at the end of the experiment had attained at least twice the size of the plants in the field. Their leaves were free from curling, and even the lowermost leaves had a bright color.

EXPERIMENT 3.—A number of plants of Early Baltimore and an unnamed determinate variety developed at the Station growing in a nearby garden were selected. The fruits of these plants had been harvested regularly at the market-ripe stage shortly after turning red. Al-

though the plants had produced an abundance of fruits, no defoliation had taken place as late as September 1, even on the determinate variety—which is ordinarily particularly subject to severe defoliation. In contrast to these plants, general defoliation was very pronounced in most commercial fields at this time. Ripe fruits were placed underneath the undefoliated vines of several plants of each of the varieties mentioned, and suitable adjacent controls were arranged. Eight days later defoliation was prevalent in all plants under which fruits had been placed but was more pronounced for the determinate variety than for Early Baltimore. More fruits were placed under the plants, and in another week defoliation had become extreme. All the leaves of both varieties had dried and abscissed, with the exception of a few at the tips of some of the branches which extended beyond the area where the ripe fruit had been placed. The plants about which no fruits had been placed remained green and did not defoliate.

Ripe fruits were also placed underneath the foliage of a so-called "bull" plant, a type of plant which is present occasionally in most large tomato fields. Such plants are usually large and vigorous, seldom produce any fruit, and do not lose their leaves even though defoliation is pronounced for other plants of the field. In the case of such vegetative, nonfruiting plants, 3 days after the fruits had been placed about them several of the leaves were yellow and curled, and at the end of 6 days a considerable number were dry. Additional ripe fruits were added later, and after about 2 weeks had elapsed practically all the leaves on the lower half of the plant had dried and abscissed. The upper leaves on the upright branches, which were considerably taller than those of normal-bearing plants, were the only ones which remained green. The leaves on this plant also had spots from which spores of *Alternaria* and *Septoria* were obtained. All other such vegetative nonfruiting plants of the field were examined and found to be entirely free of defoliation. Usually some of the leaves of these plants had a few *Alternaria* lesions, but the leaves were not discolored and the plants as a whole were free from either disease.

EXPERIMENT 4.—A number of plants in the field were stripped of all green fruits before the initiation of defoliation. These plants remained green, did not defoliate, and were relatively free from disease lesions, while adjacent plants permitted to bear fruit showed marked defoliation.

OTHER TESTS.—From the many observations it did not appear that the typical defoliation taking place in fields was brought about by a decreased supply of nutrients in the soil during the latter part of the growing period. In one trial a number of plants were supplied with solutions containing soluble and readily available nitrogen, phosphorus, and potassium. These elements were supplied singly and in various combinations at regular intervals. The plants so treated defoliated in the same manner as untreated plants whenever ripe fruits were present.

In another nutrition experiment conducted in the greenhouse a number of tomato plants were grown in a quartz gravel culture and supplied with nutrient solutions in which the levels of nitrogen, phosphorus, and sulphur varied. Nutrient solutions were applied daily during the earlier part of the experiment, followed by applications once each week, and still later once in 2 weeks. Finally nutrients were entirely replaced by daily watering. These treatments resulted in

varying responses in the different nutrient series, and after prolonged withholding of nutrients the plants in general turned pale and became hardened, but in no case was there any defoliation similar to that found in the field.

On the basis of the evidence collected to date, it is probable that gaseous emanations from ripe fruits present on the vines are a major contributing factor resulting in defoliation of tomato plants as grown under commercial field culture. On the basis of what is known about such emanations from ripening fruits of various kinds, and on additional experimental evidence, ethylene may be one of the principal compounds involved.

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VARIETAL DIFFERENCES IN ANATOMY OF CROSS-SECTION OF WHEAT GRAIN[1]

JAMES C. BATES

(WITH TEN FIGURES)

The varieties of wheat selected for this study were Turkey, Tenmarq, Blackhull, Chiefkan, and Kawvale. The grains were obtained from the Kansas Agricultural Experiment Station rust nurseries on June 4, 1941. Average-size grains were selected from the mid-portion of the spike. The grain was in the stiff-dough stage.

After shaving off the ends, the grains were treated for 48 hours with killing and fixing fluid (2 per cent chromic acid, 25 cc.; 10 per cent acetic acid, 25 cc.; commercial formaldehyde, 15 cc.; and distilled water, 25 cc.). The solution was then removed by washing the grains overnight in running water. After washing, they were treated for 2 hours in each of the following: dioxan-water, 1:2; dioxan-water, 1:1; dioxan-water, 2:1; and two changes of pure dioxan.

After dehydration, the material was transferred to a saturated solution of parawax in dioxan in the paraffin oven, 12 hours; two changes of pure parowax, 3 hours each; then transferred to Tissuemat (Fisher, M.P. 56–58) and cast into blocks. The blocks were left in water until time of sectioning. When difficulties were encountered, the paraffin was cut away at one end and the grain left in water for a few hours longer. The sectioning was done with a rotary microtome, using a safety razor blade. The paraffin ribbon was cut into pieces and floated on water at 35°–40° C. The pieces were then floated on clean slides previously treated with Szombathy's gelatin adhesive as modified by HAUPT,[2] and while still wet were exposed to the fumes of commercial formaldehyde for an hour or longer. They were then dried in the air, the paraffin removed with xylene, and mounted in hyrax without staining.

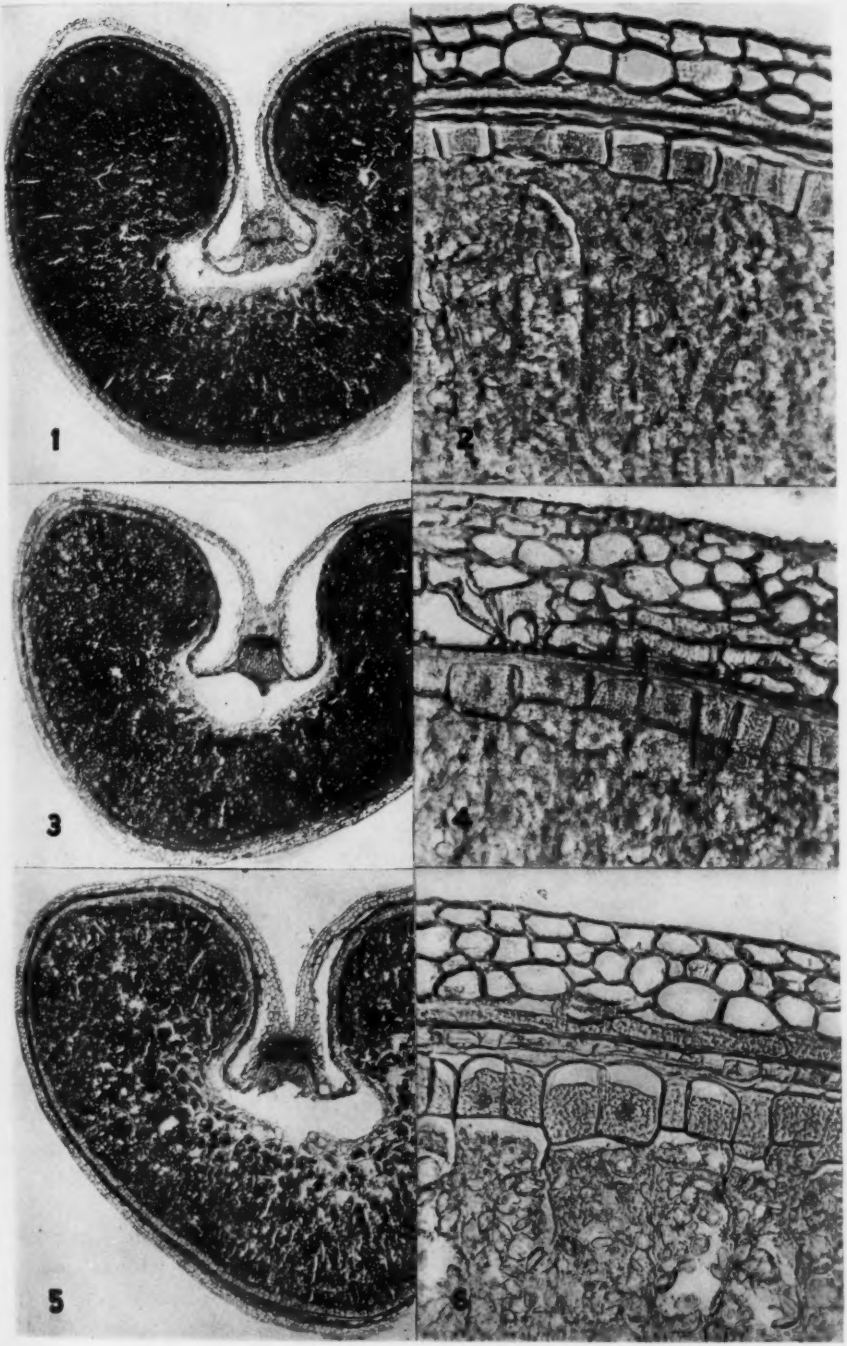
Figures 1–10 show the cross-sections and portions of the bran layer.

The data in table 1 were calculated from measurements taken from the photomicrographs. They show that although the cross-sectional area of the grain is less and the combined thickness of the bran and aleurone layers is less in Kawvale than in Tenmarq, the area of the combined bran and aleurone layers is greater and consequently the percentage cross-sectional area of starchy endosperm is less in Kawvale. This is due to the difference in the shape of the cross-section (figs. 1, 2, 9, 10).

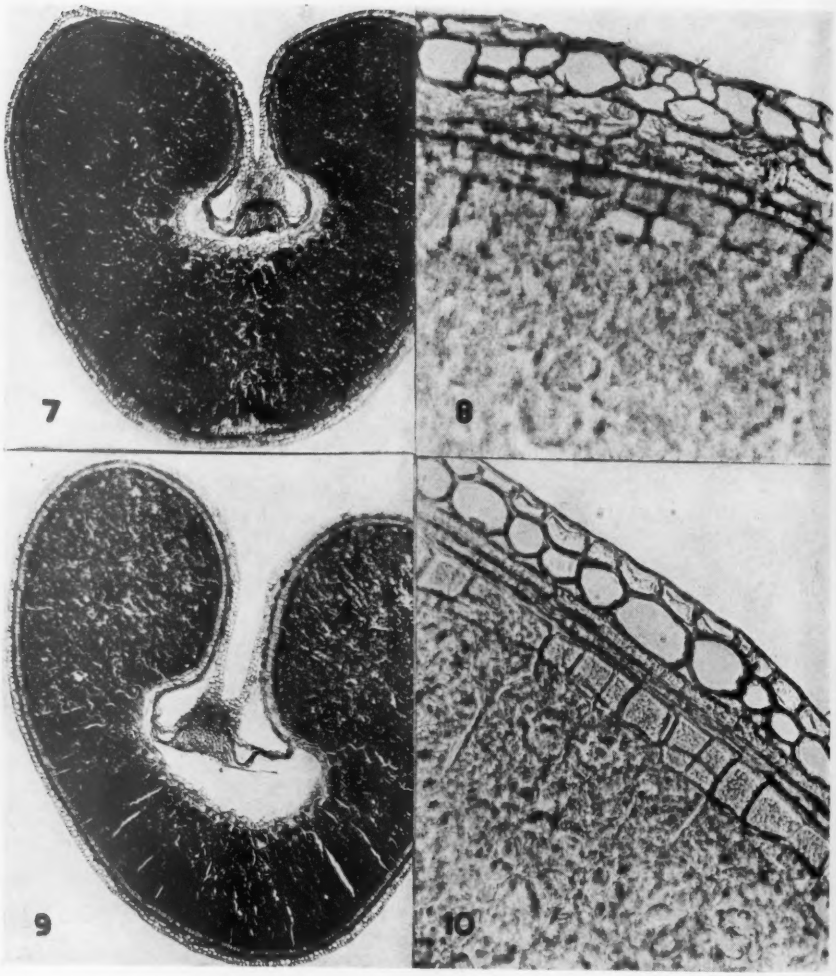
The combined thickness of the bran and aleurone layers is greater in Blackhull (figs. 3, 4) and Chiefkan (figs. 5, 6) than in the other varieties. The percentage cross-sectional area of starchy endosperm is smaller in Blackhull than in any other variety. Chiefkan has a larger cross-sectional area of grain and a larger percentage cross-sectional area of starchy endosperm than has Blackhull, although

[1] Contribution no. 434 from the Department of Botany, Kansas Agricultural Experiment Station.

[2] HAUPT, A. W., A gelatin fixative for paraffin sections. Stain Technol. 5:97–98. 1930.



FIGS. 1–6.—Cross-section of grain and section through bran and aleurone layers of varieties of wheat: figs. 1, 2, Tenmarq; figs. 3, 4, Blackhull; figs. 5, 6, Chiefkan.



FIGS. 7–10.—Cross-section of grain and section through bran and aleurone layers of varieties of wheat: figs. 7, 8, Turkey; figs. 9, 10, Kawvale.

the combined thickness of the bran and aleurone layers is about the same.

TABLE 1

ANATOMICAL DIFFERENCES IN CROSS-SECTION OF WHEAT GRAIN

VARIETY	CROSS-SECTIONAL AREA				COMBINED THICKNESS OF BRAN AND ALEURONE LAYERS (MM.)
	Grain (sq. mm.)	Bran and aleurone layers combined* (sq. mm.)	Starchy endosperm (sq. mm.)	Starchy endosperm (%)	
Hard red winter wheats					
Tenmarq....	5.91	1.32	4.59	77.66	0.106
Chiefkan....	5.96	1.71	4.25	71.30	0.143
Turkey.....	5.55	1.60	3.95	71.17	0.112
Blackhull....	4.53	1.37	3.16	69.60	0.140
Semi-hard red winter wheat					
Kawvale....	5.71	1.50	4.21	73.73	0.097

*Areas of large intercellular spaces at base of crease excluded.

Turkey (figs. 7, 8), which has frequently been used as a standard in testing wheat varieties, comes nearest to representing the average grain in this study of varietal differences.

The percentage volume of starchy endosperm which greatly influences percentage flour extraction is determined by the size and shape of the grain, combined thickness of bran and aleurone layer, and proportionate size of the germ. Since the percentage cross-sectional area of starchy endosperm is determined by the shape, area, and combined thickness of bran and aleurone layers in the cross-section, it is undoubtedly an important factor in determining the percentage of flour extraction.

Appreciation is expressed to Dr. JOHN H. PARKER, Director of the Kansas Wheat Improvement Association, for suggesting this problem.

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CURRENT LITERATURE

Upper Cretaceous Floras of the Rocky Mountain Region. II: *Flora of the Lance Formation at Its Type Locality, Niobrara County, Wyoming.* By ERLING DORF. Preprinted from Carnegie Institution of Washington Publication no. 508. October 2, 1942. Pp. 79+159. Illustrated.

The Lance flora, long a source of uncertainty concerning the Mesozoic-Cenozoic boundary, has been studied from abundant, new carefully made collections. Nearly all the earlier material was examined also, and it now seems evident that little conflict remains between age interpretations based on animal and plant fossils. Lancian age is defined as the temporal term for latest Cretaceous of the Rocky Mountain region, delimited below by true Fox Hills and above by beginning of the Paleocene.

The seventy species of plant fossils assembled from the Lance differ considerably from those known in older beds and contrast more strongly with assemblages of the early Mesozoic. At the same time all but thirteen of the Lancian species are known to occur in other latest Cretaceous strata outside the type district of the Lance formation, so that the flora affords substantial practical assistance to the geologist. The plant assemblages are ably summarized in tables and discussion. Dominant forms are chiefly dicots but include abundant *Sequoia* and *Araucarites*. Analysis suggests that the flora existed under warm temperate more nearly than under subtropical conditions in humid lowlands.

In discussing the principles and methods of correlation, DORF mentions similarity of assemblages, use of widespread short-ranging "index" fossils, and use of a widespread dominant form or forms not necessarily of restricted age. The earliest appearance of new vegetational types is preferred to the latest known occurrence of old as a datum for correlation purposes. Correlation based on the recognition of evolutionary series is not mentioned, although in other paleontological investigations the method has been particularly constructive, since it provides a more adequate explanation for the appearance or disappearance of biologic entities.

Of the seventy forms treated, forty-nine have been referred to thirty-three modern plant families, one only questionably. Fifteen modern genera, four of them questionably, are included under their respective families. Forty-seven forms are apparently generically distinct or else their correlative modern genus has not been recognized.

Probably all, or nearly all, scientists will admit the desirability of identifying modern plant groups as far back in the fossil record as they can be reliably traced. DORF has made extensive comparisons of his fossils with modern specimens, and apparently all allocations to modern genera have been made with careful discrimination. Many of these allocations, however, do not accord with the current revised code of nomenclature (1930 Cambridge revision; Gustav Fischer, Jena, 1935), which provides (p. 4) that "the nomenclature of Fossil Plants of all groups begins with the year 1820." Obviously the types of modern genera validly established before that date cannot be utilized for identification of fossils if these rules are to be followed. Needless to say this addition to Article 20 has not increased the prestige of formalized nomenclature among paleobotanists. DORF's study of the Lance flora, together with many other of the better treatments of Cretaceous and Tertiary fossils, illustrates the desirability of modifying this regulation.—JAMES M. SCHOPF.

The Carnivorous Plants. By FRANCES ERNEST LLOYD. Waltham, Massachusetts: Chronica Botanica Co.; New York City: Stechert and Co., 1942. Pp. xv+352. Figs. 556.

Biologists will welcome this interesting and comprehensive book. Thirteen chapters are given to the fifteen genera of carnivorous angiosperms. For each there is discussion of its discovery, habitat, distribution, vegetative organization, propagation, and seed formation. An additional chapter takes up certain fungi for which zoöpahgy is known. A bibliography concludes each chapter.

Special attention is given to the traps, as to type, structure, mechanism, and operation. The structural survey of these organs includes anatomical and developmental details as well as consideration of homologies. For each type there is discussion of its secretions and any experimental work relating to the nutritive utilization of the trap contents. Much information is brought together about trapped organisms and others commonly living in or associated with these structures.

All illustrations except a few text figures are grouped on thirty-eight plates, bound in the back of the book. These include both photographs and drawings, largely original, and most of them are by the author. Naturally the volume finds its climax in the chapters on *Utricularia* and related genera, since they are enriched by the author's extended researches on these forms. His critical judgment, however, has been extended to all parts of the book and becomes its strongest recommendation to botanists. —ROBERT B. WYLIE.

Fieldbook of Native Illinois Shrubs. By LEO R. TEHON. Urbana: Illinois Natural History Survey Division, Manual 3, 1942. Pp. vi+307. Illustrated. $1.25.

This book is a complement to the previously published manual describing the wild flowers of Illinois. Native and a few naturalized shrubs, including the American yew, the common juniper, several species of *Smilax*, and species belonging to forty dicotyledonous families are keyed and described. Line drawings and four color plates illustrate certain features of every species. An introductory discussion of habitats, a map of Illinois, a glossary and an index complete the book. Its usefulness will undoubtedly extend far beyond the boundaries of Illinois.—PAUL D. VOTH.